



AGRICULTURAL RESEARCH INSTITUTE

PUSA

THE NEW PHYTOLOGIST

FOUNDED BY A. G. TANSLEY IN 1902

EDITED BY

A. R. CLAPHAM, H. GODWIN, W. O. JAMES

VOLUME XXXIII

PAGES viii AND 394

WITH ONE PLATE AND NUMEROUS FIGURES
IN THE TEXT

CAMBRIDGE
AT THE UNIVERSITY PRESS

1934

PRINTED IN GREAT BRITAIN

INDEX OF AUTHORS

	PAGE
✓ BABCOCK, E. B. Basic Chromosome Numbers in Plants with Special Reference to the Compositae	386
BHÂRADWÂJA, Y. A New Species of <i>Nostochopsis</i> (<i>Nostochopsis radians</i> sp.nov.) (2 Text-figures)	I
BRYANT, A. E. A Demonstration of the Connection of Protoplasts of Endodermal Cells with Casparian Strips in Roots of Barley (Plate I)	231
CLAPHAM, A. R. Chromosomes and Plant Breeding, by C. D. DARLINGTON (Review)	172
— The Fresh Water Algae of the United States, by G. M. SMITH (Review)	393
CORNER, E. J. H. Researches on Fungi, Vol. v, by A. H. R. BULLER (Review)	318
DARBISHIRE, O. V. Die Flechten, von F. TOBLER (Review) .	389
DARLINGTON, C. D. Introduction to Cytology, by L. W. SHARP (Review)	320
GILMOUR, J. S. L. Plants and Human Economics, by R. GOOD (Review)	171
GODWIN, H. Deutsche Waldbäume und Walddtypen, von W. SCHOENICHEN (Review)	392
— La Mutation chez les Orchidées, par L. REYCHLER (Review)	392
— Pollen Analysis, An Outline of the Problems and Potentialities of the Method:	
Part I. Technique and Interpretation (9 Text-figures) .	278
Part II. General Applications of Pollen Analysis (10 Text-figures)	325
HÂRDTL, H. Ueber der Gestalt der Bäume (6 Text-figuren) .	45
HARLEY, J. L. Some Critical Experiments upon Culture Methods used for Fungi	372
HOWARTH, W. O. British Economic Grasses, Their Identification by the Leaf Anatomy, by S. BURR and D. M. TURNER (Review)	68

	PAGE
HICKS, P. A. and PANISSET, T. E. Quantitative Determination of Minute Amounts of Chlorophyll (5 Text-figures)	199
INGOLD, C. T. The Spore Discharge Mechanism in <i>Basidiobolus ranarum</i> (2 Text-figures)	274
JAMES, W. O. Dynamics of Photosynthesis (6 Text-figures)	8
— Elements of Botany, by R. M. HOLMAN and W. W. ROBBINS (Review)	69
— School Botany, by M. SKENE (Review)	323
JOSHI, A. C. Variations in the Medullary Bundles of <i>Achyranthes aspera</i> L. and the Original Home of the Species (2 Text-figures)	53
MCCALL, D. Index to the Genera and Species of the Diatomaceae and their Synonyms, by F. W. MILLS (Review)	70
MCLEAN, R. C. Substitution Staining with Free Dye Acids and Dye Bases	316
MILLNER, M. E. Anatomy of <i>Silene vulgaris</i> and <i>Silene maritima</i> as related to Ecological and Genetical Problems. I. Root Structure (19 Text-figures)	77
NORMAN, A. G. Methods of Cellulose Chemistry, by C. DORÉE (Review)	71
OLIVER, F. W. D. H. Scott	73
PAL, N. L. Studies on the Respiration of Conjugating <i>Spirogyra</i> with Special Reference to Fat Metabolism (17 Text-figures)	241
PANISSET, T. E., <i>see</i> HICKS	
PHILIPSON, W. R. The Morphology of the Lemma in Grasses (7 Text-figures)	359
SANDISON, S. Rooting of Cuttings of <i>Lonicera japonica</i> : a Preliminary Account (5 Text-figures)	211
SAUNDERS, E. R. Comments on "Floral Anatomy and its Morphological Interpretation" (50 Text-figures)	127
— Note on the Floral Anatomy of <i>Rivina humilis</i> L.	66
SMITH, J. H. Recent Advances in the Study of Plant Viruses, by K. M. SMITH (Review)	72
SMITH, K. M. Virus Diseases of Plants, by J. GRAINGER (Review)	171

Index of Authors

v

	PAGE
TANSLEY, A. G. Julius Sachs, der Begründer der neueren Pflanzenphysiologie, 1832-97, by E. G. PRINGSHEIM (Review) .	232
THOMAS, H. H. Nature and Origin of the Stigma. A Contribution towards a New Morphological Interpretation of the Angiosperm Flower (15 Text-figures)	173
THOMPSON, J. McLEAN. Comments on Recent Statements regarding the Nature and Origin of the Angiospermic Stigma	306
THOMSON, R. B. Heterothally and the Seed Habit <i>versus</i> Heterospory	41
— Seed-plant Feature of the Root in Marattiaceae (2 Text-figures)	96
TURNER, J. S. Manometric Methods as applied to the Measurement of Cell Respiration and other Processes, by M. DIXON (Review)	322
TURRILL, W. B. Correlation of Morphological Variation with Distribution in some Species of <i>Ajuga</i> (15 Text-figures and 1 map)	218
WANSCHER, J. H. Basic Chromosome Number of the Higher Plants (2 Text-figures)	101
— Secondary (Chromosome) Associations in Umbelliferae and Bicornes (6 Text-figures)	58
WEISS, F. E. Plant Chimaeras and Graft Hybrids, by W. NEILSON JONES (Review)	390

INDEX OF SUBJECTS

	PAGE
<i>Achyranthes aspera</i> L., Variations in the Medullary Bundles of, and the Original Home of the Species (2 Text-figures)—A. C. Joshi	53
<i>Ajuga</i> , see Correlation	
Basic Chromosome Numbers in Plants with Special Reference to the Compositae—E. B. Babcock	386
Basic Chromosome Number of the Higher Plants (2 Text-figures)—J. H. Wanscher	101
Bäume, Ueber der Gestalt (6 Text-figures)—H. Härdtl	45
Casparian Strips, see Endodermal Cells	
Chlorophyll, Quantitative Determination of Minute Amounts (5 Text-figures)—P. A. Hicks and T. E. Panisset	199
Correlation of Morphological Variation with Distribution in some Species of <i>Ajuga</i> (15 Text-figures and 1 map)—W. B. Turrill	218
Culture Methods used for Fungi, Some Critical Experiments upon (11 Text-figures)—J. L. Harley	372
Dye Acids and Dye Bases, see Substitution-Staining	
Dynamics of Photosynthesis (6 Text-figures)—W. O. James	8
Endodermal Cells, Connection of the Protoplasts with Casparian Strips in Roots of Barley (Plate I)—A. E. Bryant	231
Fat Metabolism, see Respiration of Conjugating <i>Spirogyra</i>	
Floral Anatomy, see <i>Rivina humilis</i> L.	
"Floral Anatomy and its Morphological Interpretation," Comments on (50 Text-figures)—E. R. Saunders	127
Form of Trees, see Bäume	
Fungi, see Culture Methods	
Heterospory, see Heterothally	
Heterothally and the Seed Habit <i>versus</i> Heterospory—R. B. Thomson	41
Lemma, see Morphology of the Lemma	
<i>Lonicera japonica</i> , Rooting of Cuttings: A Preliminary Account (5 Text-figures)—S. Sandison	211

Index of Subjects

vii

PAGE

Marattiaceae, Seed-plant Feature of the Root (2 Text-figures) —R. B. Thomson	96
Medullary Bundles, <i>see Achyranthes aspera</i>	
Morphology of the Lemma in Grasses (7 Text-figures)—W. R. Philipson	359
<i>Nostochopsis</i> , A New Species (<i>Nostochopsis radians</i> sp.nov.) (2 Text-figures)—Y. Bhâradwâja	I
Photosynthesis, <i>see Dynamics</i>	
Pollen Analysis, An Outline of the Problems and Potentialities of the Method—H. Godwin:	
Part I. Technique and Interpretation (9 Text-figures)	278
Part II. General Applications of Pollen Analysis (10 Text-figures)	325
Respiration of Conjugating <i>Spirogyra</i> with Special Reference to Fat Metabolism (17 Text-figures)—N. L. Pal	241
REVIEWS:	
British Economic Grasses, Their Identification by the Leaf Anatomy (Burr and Turner)—W. O. Howarth	68
Cellulose Chemistry, Methods of (Dorée)—A. G. Norman	71
Chromosomes and Plant Breeding (Darlington)—A. R. Clapham	172
Deutsche Waldbäume und Waldtypen (Schoenichen)—H. Godwin	392
Diatomaceae, Index to the Genera and Species (Mills)— D. McCall	70
Elements of Botany (Holman and Robbins)—W. O. James	69
Flechten (Tobler)—O. V. Darbishire	389
Fresh Water Algae of the United States (Smith)—A. R. Clapham.	393
Introduction to Cytology (Sharp)—C. D. Darlington	320
Julius Sachs, der Begründer der neueren Pflanzenphysiologie, 1832-97 (Pringsheim)—A. G. Tansley	232
Manometric Methods as applied to the Measurement of Cell Respiration and other Processes (Dixon)—J. S. Turner	322

Reviews (<i>cont.</i>):	PAGE
Mutation chez les Orchidées (Reychler)—H. Godwin . . .	392
Plant Chimaeras and Graft Hybrids (Neilson Jones)— F. E. Weiss	390
Plant Viruses, Recent Advances in the Study of (K. M. Smith)—J. H. Smith	72
Plants and Human Economics (Good)—J. S. L. Gilmour .	171
Researches on Fungi. Vol. v (Buller)—E. J. H. Corner .	318
School Botany (Skene)—W. O. James	323
Virus Diseases of Plants (Grainger)—K. M. Smith . .	171
<i>Rivina humilis</i> L., Note on Floral Anatomy—E. R. Saunders	66
Rooting of Cuttings, <i>see</i> <i>Lonicera japonica</i>	
Scott, D. H.—F. W. Oliver	73
Secondary (Chromosome) Associations in Umbelliferae and Bicornes (6 Text-figures)—J. H. Wanscher	58
<i>Silene vulgaris</i> and <i>Silene maritima</i> , Anatomy related to Ecological and Genetical Problems. I. Root Structure (19 Text-figures)—M. E. Millner	77
Spore Discharge Mechanism in <i>Basidiobolus ranarum</i> (2 Text- figures)—C. T. Ingold	274
Stigma, Comments on Recent Statements regarding the Nature and Origin—J. McLean Thompson	306
Stigma, Nature and Origin, A Contribution towards a New Morphological Interpretation of the Angiosperm Flower (15 Text-figures)—H. H. Thomas	173
Substitution Staining with Free Dye Acids and Dye Bases— R. C. McLean	316
Umbelliferae and Bicornes, <i>see</i> Secondary Associations	

THE NEW PHYTOLOGIST

VOL. XXXIII, No. 1

2 MARCH, 1934

A NEW SPECIES OF *NOSTOCHOPSIS* (*NOSTOCHOPSIS RADIANS* SP. NOV.)

By YĀJÑAVALKYA BHĀRADWĀJA

Department of Botany, East London College,
University of London

(With 2 figures in the text)

THE alga forming the subject of this communication was growing on submerged stones in a shallow stream running in a deep shady valley in the Jog Falls region of Mysore State¹. It forms hemispherical or subspherical blue-green or olive-green strata, very soft to the touch and attached closely to the substratum. From the surface of each growth numerous long threads project freely into the surrounding water (Fig. 1 A); the maximum width of a stratum, together with these threads, is about 1 cm.

The plant body consists of a system of branched filaments which are not embedded in any mucilage. It can be divided roughly into three regions, viz. (a) the small compact basal portion which is attached to the substratum and consists of densely arranged, irregularly curved and profusely branched filaments, (b) a large middle region in which the filaments are rather loosely arranged, slightly narrower and less branched, and (c) the outermost region of long, unbranched, projecting threads which are much narrower, but of uniform thickness throughout and are almost as long as the rest of the plant body, terminating in a rounded apex. In the middle region the filaments are straight or slightly curved and run more or less radially, but there is no sharp line of demarcation between this and the basal region which gradually merges into the middle one.

The filaments in the basal region and in the older parts of the middle region possess a thin hyaline sheath following the contour of

¹ I am indebted to Professor M. A. Sampathkumaran for the material of this alga.

the cells (Figs. 1 C–F, 2 A and B), but the younger filaments of the middle region (Fig. 1 B) and the outermost freely projecting threads (Fig. 1 G) are altogether devoid of a sheath. The sheath is fairly firm since it retains its cylindrical form after parts of the trichomes have perished (Figs. 1 E, 2 A and B).

There is no definite arrangement of the branches in the basal region, but in the middle region, except in rare cases, they all arise on one side of the main filament. All the branches are true. In the development of a branch the middle part of a cell becomes protruded and cut off by a wall, at first pushing out the enveloping sheath. Usually the latter does not rupture until the protuberance has grown to a certain length. The growth of the branches is apical, though some intercalary division takes place as well. The branch may later secrete a new sheath of its own throughout its length (Fig. 2 A), but this is not always the case. The young branches stand almost at right angles to the cells that bear them (Figs. 1 E, 2 C), but as the branches elongate they bend and take up a more or less radial position with respect to the plant body as a whole.

The colour of the trichomes is blue-green. In the compact basal region the cells are up to 8.4μ in diameter and usually more or less rounded, sometimes barrel-shaped, with deep constrictions at the joints (Fig. 1 C and D). The surface of contact between the cells is often quite narrow, and in many cases the cells appear connected by obvious processes, resembling protoplasmic connections. When it has been possible to obtain a clear view, however, these processes were seen to be bridged by a delicate septum, and it does not appear that true protoplasmic connections exist in this form. The cells contain a few large granules. In the middle region the cells are much elongated and narrower (up to 6.3μ in diameter), being more or less barrel-shaped in the inner (Figs. 1 E and F, 2 A and B) and cylindrical, with or without constrictions at the joints, in the outer part (Fig. 2 C). The granules in these cells are slightly smaller than those in the cells of the basal region. The cells of the projecting threads are elongate cylindrical without constrictions at the joints and possess fine granular contents. They are 1.5 – 3.1μ in diameter (average about 2.1μ) and 7 – 50μ in length. The majority of the projecting threads are healthy throughout, but some show signs of disintegration as evidenced by the possession of an almost rounded vacuole at each end of the cells and a slight constriction at the septa (Fig. 1 G). At these points the cells eventually split apart.

The heterocysts, which are always lateral in position, are formed

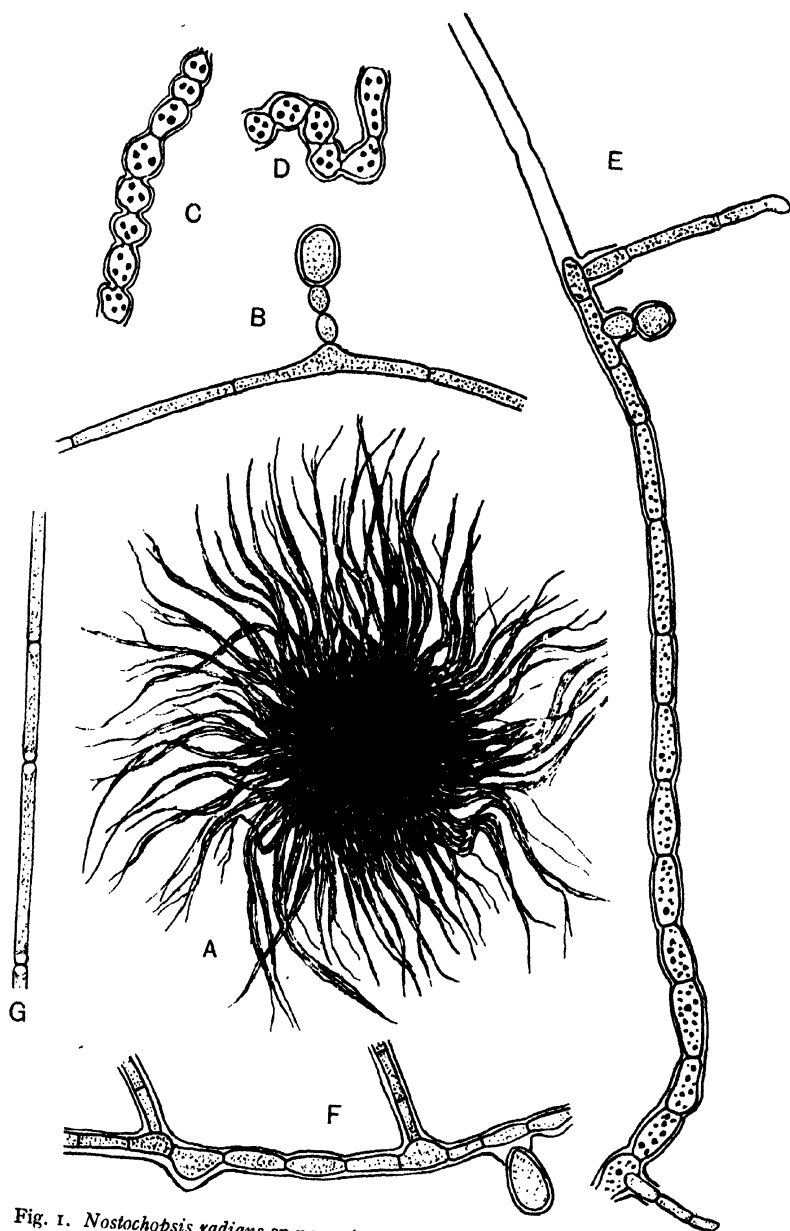


Fig. 1. *Nostochopsis radians* sp. nov. A, stratum showing habit. B, portion of young filament of middle region. C and D, portions of filaments of basal region. E and F, portions of old filaments of middle region. G, portion of projecting thread. (A $\times 10$; B-G $\times 850$.)

only in the basal and middle regions of the stratum, being more numerous in the latter. They are either sessile (Figs. 1 F, 2 F), arising directly from the filaments, or are situated at the end of a one- or two-celled (very rarely three-celled) stalk whose cells are almost spherical (Figs. 1 B and E, 2 B-E). When such heterocysts develop

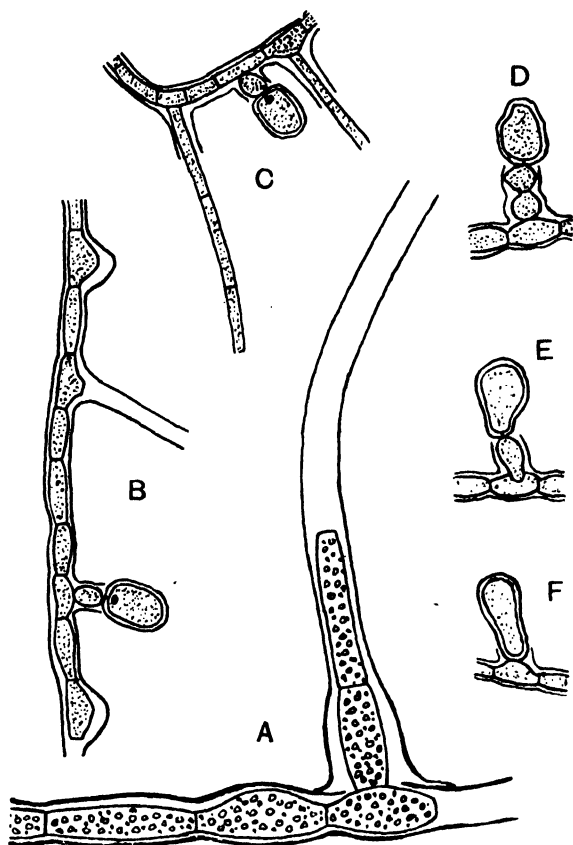


Fig. 2. *Nostochopsis radians* sp. nov. A and B, portions of old filaments of middle region. C, portion of filament from the outer part of middle region. D-F, portions of filaments with lateral heterocysts. (A $\times 1820$; B-F $\times 850$.)

upon a sheathed trichome the sheath of the latter invariably surrounds the stalk cells (Figs. 1 E, 2 B-E), but when the naked trichomes of the middle region bear stalked heterocysts the stalk may or may not be surrounded by a sheath (Fig. 1 B). The latter is secreted around the stalk cells of the heterocysts and the cell of the main filament upon which the heterocyst is borne, and also sometimes around one or two

adjacent cells in either direction. In no case does the sheath envelope the heterocyst. The heterocysts are usually unilateral, being formed on the same side of the filaments as the branches (Figs. 1 E, 2 B and C). They develop in the same way as the latter, usually arising from the middle of the cell that bears them; very occasionally they are placed a little on one side. They are usually ellipsoidal, ovate or obovate in shape and measure $4-9\mu$ in breadth and $6-15\mu$ in length. They have yellowish contents, sometimes including refractive granules.

Spores and hormogones have not been observed.

On account of its true branching, its general habit and the presence of lateral heterocysts, the alga above described must be referred to the Nostochopsidaceae of Geitler in which he includes the genera *Mastigocoleus*, *Nostochopsis*, *Myxoderma* and *Mastigocoleopsis*. It has no resemblance with *Mastigocoleus* except in the occasional presence of a thin firm sheath; it differs from *Myxoderma* in habit, in the mode of arrangement of the filaments within the plant body and in the presence of a thin firm sheath. On the other hand it resembles *Nostochopsis* in its attached mode of growth, in the radial arrangement of the filaments, in the form of the cells, and in the presence of both sessile and stalked lateral heterocysts. It does not, however, completely agree with any of the described species of this genus.

It differs from *N. lobatus* Wood in the much smaller solid thallus, in the absence of special unseptate branches (2, Fig. 358) and in the ends of the ultimate branches never being club-shaped (2, Fig. 358 and (1), Pl. VII, figs. 3 and 5). It contrasts with *N. Wichmannii* Weber van Bosse in the absence of well-marked zonation in the stratum and of intercalary heterocysts (2, Figs. 359*b* and 360). From both these species it further differs in the profuse branching of the central region of the plant body and the unbranched character of the peripheral threads (2, Figs. 357 and 359*b*), as well as in the narrow width of the latter and their greatly elongated cells. Finally it differs from *N. Hansgirgi* Schmidle in the blue-green or olive-green colour, in the absence of the intercalary meristematic zone described by Schmidle (3, p. 179) and the consequent absence of tapering or club-shaped apices on the ultimate branches, in the lack of intercalary heterocysts and in its aquatic habitat.

This alga further differs from all the described species of *Nostochopsis* in not possessing a *Nostoc*-like habit¹, in the presence of a

¹ An examination of herbarium specimens of *N. lobatus* Wood at the Natural History Museums of London and Vienna and at the Botanical Gardens of Brussels and Geneva shows that the habit of this alga is always exactly like that of a *Nostoc* (cf. (5), p. 126 and (1), Pl. VII, figs. 1 and 2).

distinct firm sheath on some of the filaments¹, and in the unbranched character of the peripheral threads which become almost as long as the rest of the plant body. These differences are well marked and distinctive, but they are scarcely sufficient to warrant the establishment of a new genus. The retention of *Myxoderma*, which differs only in habit from *Nostochopsis*, also appears open to question.

The alga is therefore to be regarded as a new species of *Nostochopsis* to be named *N. radians*.

DIAGNOSIS

Stratum hemispherical or subspherical, not embedded in mucilage but soft to the touch; blue-green or olive-green; consisting of three regions, viz. (a) a small compact base attached to the substratum and composed of densely arranged, irregularly curved and profusely branched filaments, (b) a large middle region with rather loosely arranged, straight or slightly curved, somewhat narrower and less branched filaments running more or less radially, and (c) an outermost region, almost as long as the rest of the plant body, composed of unbranched narrow projecting threads of uniform thickness. Middle and basal regions gradually merging into one another. Filaments in the basal region and in the older parts of the middle region possessing thin, firm and hyaline sheaths.

Branches true, irregularly arranged in the basal region but generally unilateral in the middle region; when young placed perpendicular to the main filament, but later bending to take up a more or less radial position with respect to the plant body as a whole.

Trichomes blue-green. Cells in basal region usually more or less rounded, sometimes barrel-shaped, with deep constrictions at the joints; much elongated and more or less barrel-shaped in the inner and cylindrical with or without constrictions at the joints in the outer part of the middle region. Cells of projecting threads elongate cylindrical with or without constrictions at the joints.

Heterocysts lateral; usually ellipsoidal, ovate or obovate in shape; formed only in the basal and middle regions of the stratum; either

¹ Wood ((5), p. 126), who established *Nostochopsis* and described *N. lobatus*, writes "No sheaths are anywhere visible." The sheath shown by Bornet and Grunow ((1), Pl. VII, fig. 4) in *Mazaea rivularioides* Born. et Grun., which is now regarded as *Nostochopsis lobatus*, does not appear to be of the firm character found in the alga here described. Figures of *N. lobatus* drawn by later workers do not show any sheath. Similarly no sheath is shown by the figures of *N. Wichmannii* drawn by Weber van Bosse ((4) Fig. 9) and Frémy ((2), Fig. 360). Schmidle(3) has described the presence of a sheath in older filaments of *N. Hansgirgi*, but here again it is more or less mucilaginous and not firm.

sessile or situated at the apices of one- or two-celled (very rarely three-celled) stalks; usually arising on the same side of the filaments as the branches.

Maximum thickness of the thallus 1 cm. Diameter of the cells (a) in the basal region up to 8.4μ , (b) in the middle region up to 6.3μ , (c) in the projecting threads $1.5-3.1\mu$ (average about 2.1μ), length $7-50\mu$. Heterocysts, diam. $4-9\mu$, long. $6-15\mu$.

On stones in a shallow stream in the Jog Falls region of Mysore State, India.

The author wishes, in conclusion, to express his great indebtedness to Professor F. E. Fritsch, F.R.S., for his guidance and criticism.

REFERENCES

- (1) BORNET, E. et GRUNOW, A. *Mazaea*, nouveau genre d'algue de l'ordre des Cryptophycées. *Bull. Soc. Bot. France*, **28**, 287. 1881.
- (2) FRÉMY, P. Les Myxophycées de l'Afrique équatoriale française. *Arch. d. Bot.* **3** (1929), Mém. 2. 1930.
- (3) SCHMIDLE, W. Ueber einige von Professor Hansgirg in Ostindien gesammelte Süßwasseralgen. *Hedwigia*, **39**, 160. 1900.
- (4) WEBER VAN BOSSE, A. *Liste des algues du Siboga*. I. *Siboga-Expeditie*, **59 a**. 1913.
- (5) WOOD, H. C. Prodrômus of study of the fresh-water algae of eastern North America. *Proc. Amer. Phil. Soc.* **11**, 119. 1869-70.

THE DYNAMICS OF PHOTOSYNTHESIS

By W. O. JAMES

Department of Botany, Oxford

(With 6 figures in the text)

CONTENTS

	PAGE
Introduction	8
Photosynthesis as a series of linked reactions	9
The stages of photosynthesis	11
Photosynthesis as a photoprocess	14
The rate of photosynthesis regarded as a heterogeneous photochemical reaction	20
The attempted application of Ohm's law	29
Diffusion resistance	30
Temperature	34
Conclusion	38
References	39

INTRODUCTION

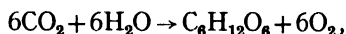
TO the modern physiologist metabolism presents itself as a complex of chemical reactions going on under peculiar circumstances. The metabolites, or plastic materials, he regards as the chemical reactants, and the peculiar circumstances are provided by the protoplasmic matrix, with its remarkable catalysts and its still more remarkable organisation. By virtue of these latter the protoplasm directs, controls, and co-ordinates the chemical activities towards ends that they would not achieve in any unorganised medium, but we are not bound to assume that it suspends the fundamental characteristics of the chemical forces: it imposes a discipline upon them but does not change their nature. In this system no single reaction is absolutely independent of any other reaction. It is claimed that no chemical reaction is entirely independent of the distribution of the fixed stars, but just as this correlation is neglected in work-a-day chemistry so we may for our convenience neglect by judicious elimination some of the connections that undoubtedly exist within the protoplasmic complex.

A metabolic process can be defined most satisfactorily as a series of chemical reactions intimately linked together, the series as a whole being in large measure self-contained; in other words the connections between reactions within a process are much closer and more important

than their connections with reactions outside it: often the latter may be disregarded altogether. The sequence is of course understood to be going on under the influence of protoplasm. According to this definition alcoholic fermentation by yeast might be regarded as a series of linked reactions such that the end-products of one reaction might become reactants in one or more following reactions. At once we want to know the nature of the different reactions involved, and one obvious method of study is to seek for and identify the various compounds formed and demolished again within the sequence. This method has, indeed, met with considerable success in the study of fermentation, but with much less when applied to photosynthesis. There remains the dynamical method, which instead of identifying substances seeks to measure the rate of the reactions in which they are concerned. Since the rate of a chemical reaction depends upon the nature of the reacting substances it is evident that the dynamical method relies for its complete application on a foreknowledge of all the reactants. Nevertheless, if only some of them are known, or if some are known and others conjectured, it may still be possible to apply this method partially but usefully. The fruitful investigation of photosynthesis up to the present time, like that of many inorganic photoprocesses, has been mainly of this kind, by measuring that is to say the rate of the process under stated conditions.

PHOTOSYNTHESIS AS A SERIES OF LINKED REACTIONS

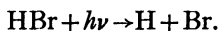
The simplest definite product of photosynthesis is a hexose sugar. The equation for the entire process is



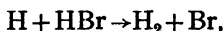
but it is quite unthinkable that carbon dioxide and water should react to give such a product at a single step. Even supposing the water to be in vast excess (which is by no means certain at the point of interaction) the reaction would be of the sixth order. That is to say, it would only occur when six carbon dioxide molecules collided simultaneously, the frequency of such collisions being proportional on the theory of probability only to the sixth power of the concentration (a fraction). Such a reaction is, in consequence, extremely unlikely to occur with measurable velocity, and in fact sixth order reactions are unknown to chemists. Dynamic studies of complex reactions generally suggest the interaction of two or at most three molecules, or in other words that the reaction is proceeding by stages, nor is there any convincing evidence that protoplasm is able to

supply the very unusual conditions that would make our process possible in a single step. In addition carbon dioxide and water do not act alone in photosynthesis; chlorophyll, light, a suitable temperature, and lesser-known factors are also essential. We should thus have to increase the order of the reaction to some unknown extent and its improbability correspondingly.

Light and heat, moreover, are rarely found to be acting in one and the same reaction, most photochemical reactions being independent of temperature changes (see Griffith and McKeown, 1929). In one of the best studied and simplest of all photochemical reactions, the decomposition of hydrobromic acid by light, it has been shown by E. Warburg that more than a single reaction is involved. The photochemical reaction achieved per quantum of light absorbed is



Each nascent hydrogen atom then reacts, without light absorption, with a further acid molecule



and two bromine atoms then unite to give bromine in the molecular form. Only the first of these three reactions involves the absorption of light; the two that follow are thermal, and such sequences are now known to be general among photoprocesses. The change studied consists usually of one simple photoreaction followed and perhaps preceded by a series of normal thermal reactions. In this respect photosynthesis is in no way peculiar.

A further argument for the existence of more than one reaction in photosynthesis is sometimes derived from the fact that when any of the controllable factors are raised to a considerable excess further increases cease to have any measurable influence on the rate of the process (see for example, Van den Honert, 1930). Thus in dim light and an atmosphere already sufficiently enriched with carbon dioxide, further additions of carbon dioxide are without effect.

The rate of a simple photochemical reaction is directly proportional to the rate at which light is absorbed and hence under very simple conditions *may* be proportional to the intensity of the illumination (the Grotthus-Draper Law). Hence in a reaction where CO_2 was changing one molecule at a time under the influence of light, we could say the rate

$$-\frac{dc}{dt} = k [\text{CO}_2] L,$$

$[\text{CO}_2]$ being the active mass of CO_2 and L the light intensity. The rate will then increase directly with an increase of $[\text{CO}_2]$ or L , at any value of the other, in contrast to the observed effect in photosynthesis.

If, however, we admit the possibility of the carbon dioxide acting bimolecularly, the whole position is changed, since the equation then becomes

$$-\frac{dc}{dt} = k_1 [\text{CO}_2]^2 L,$$

and the response of the rate to equal increases of $[\text{CO}_2]$ becomes progressively less with rising concentration until finally it becomes immeasurably small.

The force of this argument thus depends for one thing on the confidence with which we can assert that the carbon dioxide is reacting unimolecularly. Those who interpret the experimental data to mean that there is strict proportionality between carbon dioxide concentration and the rate of photosynthesis at low concentrations are perhaps entitled to rule out the bimolecular possibility. Hence they may use the subsequent deflection from proportionality as evidence that more than one reaction is in question. If on the other hand we entertain any doubt of the real existence of this proportionality, and the data are not entirely convincing, the argument has no force.

It is clearly better to think of photosynthesis as involving several chemical stages rather than one, and, in addition, there is no difficulty in realising that gaseous diffusion must be involved also. The site of the process is the chloroplast, and the source of the carbon dioxide, apart from the relatively small respiratory contribution, is the atmosphere. The air inside the leaf is not subject to any considerable draughts, i.e. mass movements, hence the movement of carbon dioxide from outside through the stomatal pores and internal atmosphere, as well as through the final watery layers, must be due to diffusion. So far from being negligible the influence of this stage on the rate of photosynthesis is often very considerable.

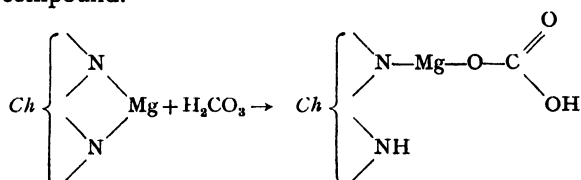
THE STAGES OF PHOTOSYNTHESIS

It will become obvious in the following pages that the general method here applied to photosynthesis is not limited to any particular series of chemical reactions. Nevertheless to have some hypothesis round which our ideas may be centred is clearly desirable, and for the sake of simplicity we shall illustrate the method by adopting a system that is possible and even plausible, though far from proved. It seems the best at present available.

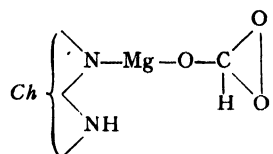
The considerations of the previous paragraph make it extremely likely that a diffusion, a photochemical, and at least one ordinary thermal ("dark") reaction are involved; and so much is now universally accepted. The complete stoppage of the process in the absence of light

demonstrates the existence of a photochemical reaction, and the high temperature coefficient under certain conditions makes it highly probable that a dark reaction is involved also; the occurrence of diffusion follows from structural considerations. The further investigation of the chemical stages has often been attempted, notably by Willstätter and Stoll (1918) and Warburg (1919, 1920). The latter now concurs in a theory first put forward by Willstätter and Stoll in 1918, which can be briefly described in the following words:

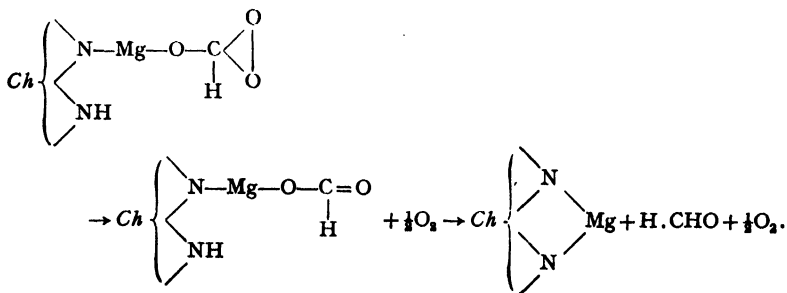
(1) The carbon dioxide arriving at the chloroplast surface is supposed to react as carbonic acid, or some simple derivative, with the magnesium of the chlorophyll molecule, forming a dissociable addition compound.



(2) Under the influence of light the compound so formed undergoes internal rearrangement of the molecule, giving an unstable compound (chlorophyll formaldehyde peroxide) in which oxygen is only loosely held.

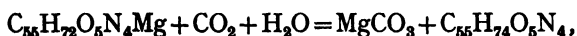


(3) This chlorophyll formaldehyde peroxide, in the presence of a peroxidase type of enzyme, readily breaks down by two stages, each releasing half a molecule of oxygen. The oxygen probably never exists in the active atomic form.



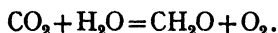
(4) Gaseous oxygen then escapes from the system and the formaldehyde polymerises eventually to sugars.

The evidence on which this hypothesis was based was derived from many experiments with chlorophyll both inside and outside the plant (Willstätter and Stoll, 1913, 1918), and is so well known that only the barest mention is needed here. Nothing could be found to suggest a chemical participation of the yellow pigments, but colloidal solutions of the green chlorophylls, *a* and *b*, were found to react with carbon dioxide even at a very low partial pressure such as that found in the atmosphere. The reaction when allowed to go to completion agreed for chlorophyll *a* with the equation



phaeophytin and magnesium carbonate being the sole products. The formation of an intermediate addition compound, which was able to dissociate back into carbon dioxide and free chlorophyll again, was shown as follows. A known quantity of carbon dioxide was added to an excess of a colloidal chlorophyll solution. The chlorophyll was then taken up in ether, ashed, and its remaining magnesium content determined. It was found that the magnesium loss by the chlorophyll was not enough for all the carbon dioxide to have formed magnesium carbonate or bicarbonate; that is to say some of the carbon dioxide remained attached to the chlorophyll molecule. When solutions containing the chlorophyll-carbonic acid compound were brought in contact with an atmosphere having only a low pressure of carbon dioxide the atmosphere gained carbon dioxide at the expense of the compound. The recovery from alcoholic solutions was quantitative, but in watery solutions a greater or lesser breakdown of the chlorophyll always took place simultaneously. In view of these facts it is unlikely that chlorophyll should fail to react with carbon dioxide dissolved in the plant, and behave merely as a photosensitiser as has been suggested; these experiments form, therefore, the basis for the first of the suggested stages.

At the other end of the sequence chlorophyll is considered to be liberated unchanged, owing to the fact that the chlorophyll content of leaves is not altered by periods of photosynthesis. The oxygen is released in equimolecular proportions with the carbon dioxide absorbed suggesting the empirical equation,



The most obvious choice for the second product is consequently formaldehyde, and much discussion has centred round this problem. It is not, however, necessary to enter into these arguments because in dynamical studies the measurement of formaldehyde is never attempted, and the measurement of subsequent products but rarely. In all that follows the reaction, which causes the liberation of free oxygen together with the formation of formaldehyde or other sub-

stances, will be considered the close of the process, the justification being solely that of convenience.

The final reaction being a release of molecular oxygen it is natural to suppose that the previous reaction has been the provision of some readily reducible substance, which is also an isomer of carbonic acid attached to chlorophyll; the most probable suggestion according to Willstätter being chlorophyll formaldehyde peroxide. Willstätter, using a horse-radish peroxidase, was unable to reconstruct this part of the process outside the plant owing to the instability of chlorophyll in watery solutions, and also perhaps because a specific oxidase is necessary. Warburg and Uyesugi (1924) compared the effect of narcotics on the dark stage of photosynthesis in *Chlorella* with their effect on the breakdown of hydrogen peroxide as carried out by the same organism. They found a very close resemblance, and naturally concluded that the reduction of a peroxide as supposed by Willstätter is likely to be a part of photosynthesis. This conclusion was further supported by the identity of the temperature relations of the two reactions discovered by Yabusoe (1924).

PHOTOSYNTHESIS AS A PHOTOPROCESS

For a quarter of a century the study of photosynthesis has been dominated for better or for worse by the "Law of Limiting Factors." The service rendered by its enunciation in 1905 was to lift the subject at a step from the sterile contemplation of "cardinal points" and to provide a practical method of analysis of its varied phenomena: every dynamical study of any importance since that date has made use of these methods. The familiar statement runs: "When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor."

The principle for the isolation of the effects of an individual factor for detailed study is, therefore, to arrange that all other factors are high in their effective ranges. In spite of its well-tried and proved utility this botanical epigram is an empiricism and, as it seems to me, not a sound one: since it presents an unnaturally angular picture. It has been realised for some time that the "law of limiting factors" could not be valid if the factors were all acting on the same reaction (Briggs, 1920), and it has been suggested that each external factor controls its own reaction, and that reaction held down to the slowest rate brings the others down to its own speed. Van den Honert (1930) goes even further: having said "I do not want to discuss this question here, but for the present I will merely assume Blackman's formula to be a true description of the facts," he goes on to cite the principle as evidence to prove the existence of serial reactions.

The "law of limiting factors" expresses the facts approximately when differences of rate are large, and since these conditions may be fulfilled experimentally it is a useful laboratory guide. When two or more factors tend to produce reactions of comparable rates it fails completely. The dependence of the rate of the sequence on the rates of all the contained reactions is, indeed, well known to chemists: Senter (1930) in a familiar text-book expresses it thus: "It is important to remember that when one of the reactions is very slow compared with the others, good constants corresponding with the slow reaction are obtained, but when the rates are not very different, the observed velocity does not correspond with any simple order of reaction."

Little attention has been paid to the formal objection to limiting factors even by authors who have attempted to consider photosynthesis from the standpoint of chemical dynamics (see, for example, Van den Honert, 1930; Maskell, 1928*a* and *b*). A good deal of criticism, derived directly from experimental results, has, however, accumulated in recent years notably that of Harder (1921) working with *Fontinalis antipyretica*. A general inspection of the curves of Fig. 1 will show that the increment of rate does not follow the increase of the factor until suddenly interrupted by the influence of some other factor in any single case except the original results of Blackman and Smith. There is always a continuous falling away from proportionality.

Much of the experimental evidence might, it is true, be put aside on the plea that the illuminated chloroplasts were not all behaving identically, were not all receiving equal light intensities or getting equal concentrations of carbon dioxide. Small deviations from an ideally sharp transition might readily be explained in these ways if there were any need to do it, and if the deviations were the only discrepancy between the facts and the theory. Such arguments have, indeed, been pressed to the limits of their acceptability by various authors (e.g. Van den Honert, 1930; Schroeder, 1924) who have sometimes ignored more important discrepancies in their results. (Cf. p. 29.) Adjustments such as these would, moreover, only be useful if the hypothesis rested on a firm theoretical basis, and appear to have no point in the present connection.

The slow attrition of the original statement under the influence of the experimental discoveries is, perhaps, best shown in the writings of its originator and his pupils (original author's *italics*).

We start this section with the following axiom. *When a process is conditioned as to its rapidity by a number of separate factors, the rate of*

the process is limited by the pace of the "slowest" factor. (F. F. Blackman, 1905.)

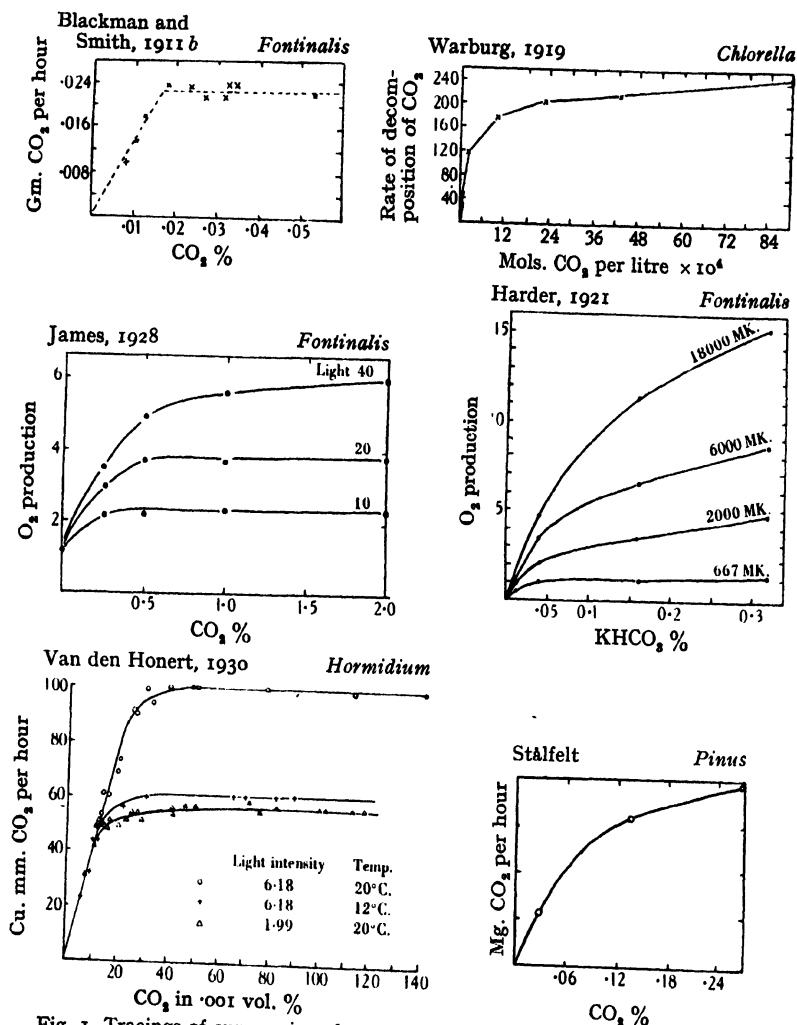


Fig. 1. Tracings of curves given by various authors representing their experimental results for the relation of photosynthetic rate to external carbon dioxide concentration.

This principle may be formulated as follows: *When several factors are possibly controlling a function, a small increase or decrease of the factor that is limiting, and of that factor only, will bring about an alteration of the magnitude of the functional activity.* (Blackman and Smith, 1911 b.)

It is conceivable, and is indeed probable, that when, so to speak, two factors are close to the limiting value a change in the one not limiting may have some appreciable effect on assimilation. (Smith, 1919.)

The subsequent history of the conception has followed a course unfortunately too common in biological science.

The "first approximation" has been widely misconstrued as a rigid law. (Maskell, 1928 *b*.)

In attempting to study the rate of photosynthesis there seems no reason why one should depart from the methods employed in the study of all other photoreactions; that is to say, one should rely on the normal methods of chemical dynamics as applied to light sensitive processes. If photosynthesis took place, or could be induced, in homogeneous solutions there would be less difficulty in formulating the relations to be expected between the various external factors and the rate on the basis of Willstätter's theory. Unfortunately even the simplest alga presents a structure of awkward complexity, and all efforts to bring about the process in homogeneous or even colloidal solutions have failed utterly. The difficulties introduced by the element of complex structure are twofold; it becomes impossible to gauge satisfactorily the diffusion path or to predict the degree of absorption of the incident light. If it were possible to deal with a system in which the chloroplast presented a plane surface to the incident light and in which the distance of the source of carbon dioxide from this surface was determinable, both difficulties would disappear. It is, therefore, interesting to consider what systems, if any, are available that approach this ideal.

The essential requirements for a formal analysis are a simple diffusion path and a simple configuration of the absorbing surface. In the first the length and cross-section must be measurable and the cross-section must also be uniform in order that Fick's equation, or some suitable derivative, may be applied.

For a plane surface with homogeneous dispersion of the absorbing material the absorption of light is given by Beer's equation

$$I_x = I_0 - c i l \quad \dots\dots(1),$$

where I_x = intensity at a distance l below the surface; I_0 the incident intensity; c the concentration of the absorbing material, and i its specific coefficient of absorption. For a photochemical reaction obeying Einstein's law (see p. 19), and so arranged that differences of concentration due to diffusion, etc. within the actual reacting system may be ignored, the relation between the incident light intensity and

the rate of the reaction is given by Plotnikov on the basis of Beer's equation as follows:

$$I_0 i k t = i p x + \log_e \frac{1 - e^{-i p a}}{1 - e^{-i p (a-x)}} \quad \dots\dots(2),$$

where x = number of mols transformed in unit volume, a = initial concentration, p = depth of reacting solution, k = reaction constant. Other symbols as before.

If absorption is limited to the surface layer because it is very intense, i.e. i is large, the second term on the right hand side of the equation approximates to $\log_e 1$ and may be neglected.

The equation then becomes

$$I_0 = \frac{p x}{k t} \quad \dots\dots(3),$$

and there is direct proportionality between the incident intensity, I_0 , and the rate, x/t . A similar relation also holds when the light absorbent is spread very thinly so that absorption is limited to the surface layers, there being no others to absorb.

No living system fulfils all these requirements exactly, and the typical dorsiventral aerial leaf cannot be considered in these terms at all owing to the complicated distribution of its chloroplasts. A lamina or filament having only a single layer of cells and flat chloroplasts arranged in a simple manner would come much nearer the ideal. Thalli such as those of *Monostroma* may be so considered and have been used by Reinke (1886) for experiments on light absorption. Wurmser (1921) has used *Ulva* thalli that are two cells thick for assimilation experiments, and there is abundant work with submerged aquatics having unicellular laminac, such as *Fontinalis antipyretica*, or bicellular as *Elodea canadensis* (Blackman and Smith, 1911 *a* and *b*; Harder, 1921, 1923; James, 1928, etc.). None of these can be supposed, however, to offer a single chloroplast layer to the incident light, and probably the best arrangement from this standpoint yet achieved is that used by recent Dutch workers (Van den Honert, 1930; Van der Paauw, 1932). This makes use of a unicellular layer of a filamentous alga, *Hormidium flaccidum*, illuminated as uniformly as possible. The system is not absolutely satisfactory on account of the cylindrical form of the cells and the curvature of the solitary chloroplasts. The latter only partially line the cell walls but may lie at varying angles. The diameter of the cells is given by Van den Honert as about 8μ , and the chloroplasts lie about 2μ below the external cell surface. The error made in assuming a uniform illumination of the chloroplast

and absorption of light in a shallow surface layer in a system such as this cannot be very great, and equation (3) may be tentatively applied. The same author was also able to make a reasonably good estimate of the area and length of the diffusion path, since the filaments were submerged only in a film of water, and a rapid stream of gas moved over their surface.

It is assumed above that we may expect the photoreaction of photosynthesis to obey the Einstein law of equivalence, that is to say that each molecule of the actual photoreactant will absorb one, and only one, quantum of light energy. This rule has justified itself in the investigation of a large number of relatively simple reactions, particularly those dealing with the activation or dissociation by light of a diatomic molecule. It applies only to the primary light reaction, and the final result of the process may appear to suggest entirely different relationships owing to the behaviour of the accompanying thermal reactions, the absorption of light by inactive substances, and so on. The validity of this relationship has not been experimentally established for the activation of any large polyatomic molecule, such as that of a chlorophyll-carbon dioxide compound, and it is conceivable that in such molecules activation may consist of the addition of energy in separate quanta perhaps to different parts of the molecule. This matter can only be settled by an experimental determination of the quantum efficiency, i.e. the ratio of the number of molecules actually caused to react per unit of absorbed energy to that predicted by the Einstein rule, or, in other words, the actual number of molecules caused to react by each absorbed quantum. In photosensitised reactions it is usual to express this efficiency in terms of the initial reactant, which in photosynthesis is carbon dioxide. The equations of p. 12 show that the quantum efficiency predicted for photosynthesis by the Willstätter sequence appears to be 1, since every light sensitive molecule contains only one molecule of carbon dioxide. The heat of combustion of formaldehyde is, however, of the order of 112,000 cal. per mol of carbon dioxide produced, and, assuming no other energy source contributes, the energy supplied by light to reverse the process must be equal to, and perhaps considerably greater than, this: how much greater could only be determined by a complete knowledge of the intermediate reactions. The size of the largest quantum in the visible spectrum (in the violet) is such that the energy supplied to a gram molecule at one quantum per molecule would not exceed about 90,000 cal. and would be less in other regions of the spectrum. Since the heats of combustion of the intermediate compounds are unknown

it is impossible to calculate exactly how many quanta must be supplied in the photochemical reaction to make the reaction go, or reciprocally its quantum efficiency. An experimental determination has been attempted by Warburg and Negelein (1922, 1923) with suspensions of *Chlorella*, and they suggest maximal quantum efficiencies varying from 0.267 to 0.213 for different wave lengths. Briggs (1929) made measurements on land leaves that he interprets as indicating an even lower efficiency. Owing to practical difficulties these determinations must not be taken too literally, but making all allowances there seems little likelihood of the true quantum efficiency being as high as unity. This means that more than one quantum per carbon dioxide molecule must be absorbed during the course of the process, and the number according to the evidence may be as high as five. Assuming that there is only one light sensitive reaction in the sequence, and that the activation of the chlorophyll-carbon dioxide molecule, therefore, requires the simultaneous impact of all the n quanta, the rate of the reaction would no longer be proportional to the incident light intensity as in equation (3) but to its n th power (I_0^n). If, on the other hand, the energetic effects were cumulative, i.e. if there were really n successive photoreactions, each involving the absorption of a single quantum, the rate of the sequence would be proportional to I_0/n , and the linear relation between intensity and reaction velocity would remain. On the whole this seems the simpler assumption in a case where orthodox photochemistry has little help to offer. It also fits in with the belief, general among botanists, that at low light intensities the curve relating rate of photosynthesis to the intensity of incident light is approximately a straight line (Stiles, 1925). The alternative assumption is, of course, at variance with this.

THE RATE OF PHOTOSYNTHESIS REGARDED AS A HETEROGENEOUS PHOTOCHEMICAL REACTION

In describing photosynthesis we may confidently assert that it begins with an uptake of carbon dioxide and that oxygen is eventually given out. We may also consider hypothetically that chlorophyll carbonic acid and chlorophyll formaldehyde peroxide are formed at intermediate stages. The actions suggested are:

- (1) Diffusion of carbon dioxide to the reactive surfaces.
- (2) A reversible reaction leading to an equilibrium between carbonic acid and chlorophyll on the one side, and chlorophyll carbonic acid on the other.

(3) The activation of chlorophyll carbonic acid by sunlight to chlorophyll formaldehyde peroxide.

(4) The enzymatic breakdown of chlorophyll formaldehyde peroxide to free chlorophyll, oxygen and other products.

This scheme, while admittedly hypothetical has sufficient plausibility to make it a useful basis for dynamical studies. It is hard to believe that the stages involved are less numerous or simpler, and what we have to do is to find how far so simple a scheme agrees with the known facts about photosynthetic rates. It will be soon enough to adopt more complicated theories when the facts drive us to it.

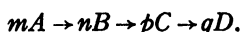
According to Willstätter's theory the chemical reactions of photosynthesis form a consecutive series in which the product of the first reaction is a reactant in the second and so on. There are at least three reactions involved, and a fourth is highly probable: two at least of the reactions are of bimolecular or higher order and one is photochemical. This is the system whose dynamics is studied, consciously or otherwise, in every investigation of the rate of photosynthesis.

The general investigation of the rates of consecutive reactions is unfortunately very complicated, and has indeed been called "one of the most difficult departments of chemical dynamics" (Mellor, 1904). The situation is usually illustrated from the behaviour of two consecutive unimolecular reactions and left at that, and the general treatment of such a sequence as photosynthesis is at present quite impossible.

It should be remembered, however, that the aim of the physiologist interested in this problem is to predict the rate of the process (= whole series of reactions) under conditions realisable in practical experiments, and the physiological problem differs from the general chemical problem, in a respect that happily makes it more open to investigation. In any chemical reaction proceeding *in vitro* the rate is continually changing as the primary reacting materials disappear and are replaced by the products, and the chemist in considering the rate of his reactions is obliged to take into account the whole range of different instantaneous velocities. While this causes no serious difficulty in dealing with an isolated reaction, it hopelessly complicates the treatment of numerous consecutive reactions, since each reaction differs from the others. In photosynthesis, on the other hand, the natural supply of the initial reactant, carbon dioxide, is unlimited, and in experimental work a constant concentration as well as uniformity of other controlling factors is always aimed at. Under these conditions it may be shown that after an initial period of adjustment

not only does the rate of the process remain constant, but also that the rates of all the included reactions become and remain identical.

The argument is as follows. Consider the series of reactions



From the law of mass action the rate of the reaction $A \rightarrow B$ is proportional at any moment to the active mass of A ; that is to say, if there is no dissociation, to its concentration $[A]$, raised to a power numerically equal to the number of its molecules in the equation, so that

$$-\frac{dA}{dt} \propto [A]^m = k_1 [A]^m.$$

Similarly

$$-\frac{dB}{dt} = k_2 [B]^n.$$

As long as the rate of the reaction $A \rightarrow B$ is greater than the rate of the reaction $B \rightarrow C$, the concentration of B will increase, so that eventually the expression $k_2 [B]^n$ will become equal to $k_1 [A]^m$. At this the two reactions have equal velocities. If, however, the rate of the first reaction is fixed by a constant renewal of A the rate of the second will necessarily remain fixed at this point also, and similar adjustments will go on in the reactions that follow after.

If our attention is limited to this *steady state* of the process it greatly simplifies the task of forecasting the effect of the various factors on the velocity in so far as it is decided by purely chemical causes alone. This necessary condition is realised in nearly all the existing experimental results. Measurements applying to the initial induction phase are comparatively rare (but see Briggs, 1933), since it occupies a relatively short time, and nearly all experimenters have gone out of their way to show that the rates they measured were maintained over long periods. They usually did this to make it plain that their experimental treatment had no injurious effect upon the plant, but it is equally important from the other point of view.

A few recent workers (e.g. Kostychev, 1931; Montfort and Neydel, 1928; Arnold, 1931) have been disturbed by the fact that their results have shown considerable irregularities when all experimental conditions were kept constant, and no obvious internal change such as movements of stomata, accumulation of end products, or shifting of chloroplasts, were observable to account for the fluctuations. It is not always clear, however, that the fluctuations did not arise through deficiencies of technique (see Boysen-Jensen and Müller, 1929; Van der Paauw, 1932) and the weight of the evidence is so heavily against these authors that their results certainly cannot be taken as typical.

To assume that photosynthesis is dependent on nothing but the mysterious complexities of the protoplasmic system, as seems to be suggested, would be to put the clock back half a century or more.

The first step in our theoretical treatment is to write velocity equations at the steady state for each of the reactions named on p. 20, introducing all the known factors. All these expressions can then be equated to one another, and an attempt made to find the relation of each of the factors to the steady rate¹.

(1) *The diffusion stage.* The rate of diffusion through a given cross-section when the rate is steady is proportional to the difference of concentration at the two ends of the diffusion path, and inversely proportional to a resistance characteristic of the medium in which diffusion is going on. For convenience the cross section of the diffusion path may be taken as unity.

If C_a is the concentration of carbon dioxide in the supplied air, C_t the concentration at the chloroplast surface, and d the resistance, the expression required for the rate at which carbon dioxide passes is, with suitable adjustment of units,

$$R = \frac{C_a - C_t}{d}.$$

This expression is valid for the carbon dioxide taken in from outside; in addition there is the carbon dioxide taken up from respiration. This quantity may be supposed to remain more or less constant, so that it may be considered simply as r and the equation for the rate of supply of carbon dioxide written

$$R = \frac{C_a - C_t}{d} + r.$$

(2) *The formation of the chlorophyll-carbonic acid addition compound.* The rate of formation of the chlorophyll carbonic-acid compound from its two constituents is determined by the concentration of carbon dioxide at the chloroplast surface, and the concentration of free chlorophyll. Assuming, with the theory, that one molecule of carbonic acid, derived, of course, from one molecule of carbon dioxide, unites with one molecule of chlorophyll, from the law of mass action we have

$$R = k_2 C_t P,$$

P being the concentration of free chlorophyll.

Since the reaction is described by Willstätter as being reversible

¹ In 1926 I had the pleasure of listening to a series of lectures by Mr G. E. Briggs in which this method was employed. The following treatment differs considerably, however, from that developed then.

we must take into account the back reaction whose velocity is given by the expression, $k_3 P_c$, P_c being the concentration of the addition compound. The net rate of the formation is, of course,

$$k_2 C_i P - k_3 P_c.$$

(3) *Activation of the chlorophyll-carbonic acid compound to chlorophyll formaldehyde peroxide.* This reaction depends upon light, and making the assumption of p. 20 we may write

$$R = k_4 P_c L,$$

L being the incident light intensity.

Allowing for the possibility of the reaction being reversible, and writing P_i for the concentration of chlorophyll-formaldehyde peroxide we have a net rate of activation

$$k_4 P_c L - k_5 P_i.$$

(4) *Breakdown of chlorophyll formaldehyde peroxide.* This occurs under the influence of a peroxidase. The simplest possible assumption as to the rate of the reaction would be based on the Michaelis rule, and would suppose the following reactions to be taking place.

Peroxide + peroxidase \rightleftharpoons peroxide-peroxidase compound.

Compound \rightarrow peroxidase + products.

Assuming that the catalyst obeys the Michaelis-Menten rule, i.e. that the first reaction is relatively fast, the net rate would be given by the expression

$$R = \frac{k_6 E P_i}{k_m + P_i},$$

when k_6 is the velocity constant of the breakdown of the enzyme substrate compound and k_m the Michaelis constant, that may be regarded as the dissociation constant of the enzyme substrate compound. Over certain ranges of concentration the rate will be proportional to the product of the enzyme and peroxide concentrations, but if we assume that this still simpler case holds in general for photosynthesis, it eventually leads to the conclusion that the rate is proportional to the total chlorophyll content. This, it is well known, is contrary to the evidence. It may well be that the Michaelis rule is too simple for the present case; but until this is shown to be so the simple expression may stand.

Since at the steady state the net velocity of each of the four stages is equal we may write

$$R = \frac{C_0 - C_i}{d} + r = k_2 C_i P - k_3 P_c = k_4 P_c L - k_5 P_i = \frac{k_6 E P_i}{k_m + P_i} \dots\dots(4).$$

It has already been shown that the intermediate concentrations P , P_o and P_i are dependent on the neighbouring reactions, and as they cannot at present be measured, we cannot arrive at a theoretical estimate of R , and its relations to C_o , L , etc., unless we can replace them by some measurable quantity. This would be afforded for example by total "chlorophyll" content (the sum that is to say of all the chlorophyll compounds present). When, however, each of the compounds is calculated in terms of this total content the velocity equation becomes exceedingly complex, and it is time to consider certain simplifications that may be introduced.

It has long been realised in practice that the relationship between the rate and the intensity of any controlling factor is only of interest when the latter is kept low. At higher values the rate becomes independent of the factor. In order to obtain the relation of the rate to the external carbon dioxide concentration, or to the light intensity, it is necessary to take advantage of this fact. A most interesting case both in theory and experiment is that where carbon dioxide concentration (C_o) or light intensity (L) is kept low, and temperature is high in relation to both. A great deal of experimental work has been carried out under these conditions, and it is, therefore, a specially favourable case for theoretical analysis.

Under the stated conditions the diffusion and photochemical reactions tend to be slow, while the combination of carbon dioxide with chlorophyll and the final destruction of the peroxide tend to be fast. Hence we may suppose that the fast breakdown reaction secures practically all the chlorophyll-formaldehyde peroxide, and the rate of the hypothetical back reaction becomes vanishingly small. The rapid combination of carbon dioxide and chlorophyll is followed by the slow photochemical reaction, hence we may assume that the former goes very nearly to its equilibrium being only slightly disturbed by the slow reaction that follows. In other words we shall make no serious error if we assume that the concentrations in that reaction are those of its equilibrium, i.e.

$$\frac{C_i \times P}{P_i} = \frac{k_3}{k_2} = K',$$

K' being the equilibrium constant, or the dissociation constant of the chlorophyll-carbon dioxide compound, whence

$$P_o = \frac{C_i P_T}{K' + C_i},$$

where P_T = total concentration of chlorophyll compounds ($= P + P_o$).

Then from the equations

$$R = \frac{C_e - C_i}{d} + r = k_4 P_e L - k_5 P_i,$$

by substituting for P_e , eliminating C_i , and neglecting $k_5 P_i$ since this should be vanishingly small,

$$C_e = \frac{RK'}{k_4 LP_T - R} + d(R - r) \quad \dots\dots(5),$$

whence we may plot the relation of R to C_e and L .

This equation is similar in form to that deduced by Maskell (1928*b*) on the basis of Warburg's "acceptor" theory of photosynthesis; the principal difference being the significance to be attributed to the

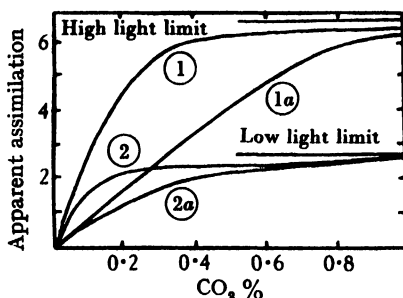


Fig. 2. Tracing of Maskell's curves for the relation between assimilation rate and external carbon dioxide concentration with a fixed value of K' . 1, with high light intensity and low diffusion resistance. 1*a*, with high light intensity and high diffusion resistance. 2, with low light intensity and low diffusion resistance. 2*a*, with low light intensity and high diffusion resistance.

symbol K' . Here it is the dissociation constant of the chlorophyll-carbon dioxide compound; whereas in Maskell's equation it represents "the ratio of the velocity constant for the dissociation of some photochemical product . . . to the velocity constant for the combination of this photochemical product with CO_2 ." The interest of the dissociation constant of the chlorophyll-carbonic acid compound in this connection has already been pointed out by Van den Honert (1930) but he fails to make clear the limitations that are implied.

Maskell has plotted the curves yielded by his theoretical equation deriving values of d , K' , etc., from his experiments with cherry laurel leaves at different seasons of the year. Fig. 2 reproduces some of the curves thus obtained for the relation between assimilation rate, and the external concentration of carbon dioxide. Different curves are given for varying values of light intensity, diffusion resistance, and

also of K' , and show the extent to which the curves may be affected by these variables. The curves may, of course, be plotted with light intensity instead of carbon dioxide concentrations as abscissae, but this only expresses the same facts in different form; it is sufficient for our purpose to take the former rendering. Fig. 1 shows a selection of experimental results obtained by various authors, often using widely different methods, for the relationship between carbon dioxide concentration and assimilation rate. The differences between these results puzzled physiologists for many years, and some authors (e.g. Romell, 1926) have not hesitated to accuse others of being unduly biased by theoretical preconceptions when plotting their curves.

It will be clear, however, when Maskell's theoretical curves are compared with the experimental curves of Fig. 1 that the different forms of the latter may readily arise, merely by variations in the values of d and other variables whose magnitudes depend upon the nature of the plants used and the conditions of the experiment. It may be objected that Maskell's curves are based upon a theory of

TABLE I

Rate of photosynthesis by Hormidium flaccidum at 20° C.

$L = 1.99$; $A (=k_4LP_T) = 58$; $K' = 1.21$.

C_0	Found	R			
		$d = 0.2285$		$d = 0.18$	
		Calculated	Difference	Calculated	Difference
8	31	29.6	-1.4	34.6	+3.6
11	42	38.1	-3.9	42.6	+0.6
12	49	—	—	44.5	-4.5
13	49	42.5	-6.5	46.1	-2.9
13	51	42.5	-8.5	46.1	-4.9
16	48	47.2	-0.8	49.5	+1.5
17	51	48.2	-2.8	50.3	-0.7
21	49	51.3	+2.3	52.5	+3.5
24	51	52.7	+1.7	53.6	+2.6
26	54	—	—	54.0	0.0
28	52	53.9	+1.9	54.4	+2.4
30	51	54.3	+3.3	54.7	+3.7
42	54	55.7	+1.7	55.9	+1.9
42	56	55.7	-0.3	55.9	-0.1
47	57	56.0	-1.0	56.2	-0.8
51	57	—	—	56.3	-0.7
72	59	—	—	56.9	-2.1
76	55	—	—	57.1	+2.1
83	57	57.0	0.0	57.1	+0.1
100	57	—	—	57.2	+0.2
102	57	—	—	57.3	+0.3
113	57	—	—	57.3	+0.3
118	57	—	—	57.4	+0.4

the intermediate stages of photosynthesis that has now been abandoned even by its author, but we have been at some pains in the foregoing pages to show that precisely the same form of equation is deducible from the more acceptable theory of Willstätter and Stoll. Mere visual comparison of curves derived from different sources may, however, be very misleading, and cannot in itself be regarded as conclusive. A more stringent test can be applied by calculating the

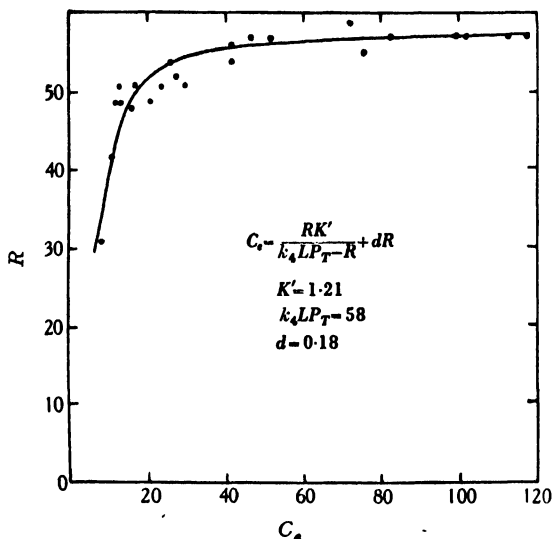


Fig. 3. Comparison between calculated values for the rate of photosynthesis and the experimental results of Van den Honert. Calculated values are represented by the continuous line and the experimental values by dots. Details of calculation and meaning of symbols in the text.

theoretical rates required by the equations for any given set of conditions, and comparing these with the values actually obtained by experiment. The results of such a test of equation (5) are shown in Fig. 3 and Table I. For a second example see also Fig. 5, p. 33.

The experimental results are taken from Table XXII of Van den Honert's paper (1930) since they appear to be the most satisfactory record available for the conditions required. External carbon dioxide concentration (C_e) and the calculated rates (R) are expressed in the units used by him. By an ingenious method Van den Honert estimated the length of the diffusion path in his experiments to be 8μ from which the diffusion resistance (d) when calculated in the appropriate units = 0.2285. No value is given for the respiratory contribution of carbon dioxide (r), but as Van den Honert was measuring relatively fast rates

of photosynthesis, and gives the rate in carbon dioxide free air as nil, r may be neglected. Values of the constants K' and A ($=k_4LP_0$) were obtained by successive approximations so the curve obtained is not necessarily the best possible fit to the data: it is intended only as a demonstration that a close fit between theory and experiment has been obtained. It will be seen from Table I that the fit is appreciably improved by taking a value for d about 20 per cent. lower than Van den Honert's estimate. His method is liable to considerably greater error than this.

The comparison shows that the experimental results can be predicted with reasonable accuracy by the equation, the deviation, even including the extremes of duplicated values, is never as much as 10 per cent. Speaking of these same curves Van den Honert said they "show a nearly ideal Blackman scheme," though this would imply an error of 20 per cent. in some of the recorded rates. In the same table results are also given for a light intensity approximately three times ($6.18/1.99$) greater than the one already treated. According to the limiting factor rule or to our present equation, which agree in the limit, this should give a maximal rate three times the maximal rate at the lower intensity. The actual ratio found was 100/57. This discrepancy apparently escaped Van den Honert's notice when he regarded the results as nearly ideal. The explanation does not lie, of course, in a failure of the rules, but in the interference at the faster rates of a third factor, perhaps temperature, unnoticed by the experimenter. In other words the simplified equation (5) that supposes the effects of such a factor to be negligible can no longer be applied; it gives, in fact, maximal values much higher than the experimental as is to be expected.

THE ATTEMPTED APPLICATION OF OHM'S LAW

Diffusion and chemical reaction both belong to the class of processes in which velocity, not acceleration, is proportional to the force acting. They may, therefore, be considered to obey the simple rule

$$\text{velocity} = \frac{\text{potential difference}}{\text{resistance}},$$

usually called Ohm's Law (see Nernst, 1923). The expression used above for the rate of diffusion depends upon this relation, and the idea of treating the chemical stages of photosynthesis in the same way is very attractive, since the expression for the rate would then simply become

$$R = \frac{P_1 + P_2 + \dots + P_n}{d_1 + d_2 + \dots + d_n}.$$

The attempt has been made by Romell (1926), and more tentatively by Maskell (1928*b*). Unfortunately there is a pitfall set here for the unwary; Romell, for example, says outright that he regards chemical potential as synonymous with concentration of the reactant; Maskell is more cautious, but appears to imply that the potential of the chemical stages is proportional to the differences of carbon dioxide concentration. Now this is clearly an error; chemical potential, or affinity, must depend on the nature, not on the quantity, of the reacting materials. It is the intensity factor of chemical energy, whereas the concentration of reacting material is the capacity factor. Neither of these is quantitatively dependent on the other. The views at present held about the nature of chemical reaction tend, moreover, to discredit this method of approach altogether, since velocity of reaction is considered to depend on intermediate "reactive states" and not upon initial and final states in terms of which "affinity" is usually measured (Hinshelwood, 1933).

DIFFUSION RESISTANCE

The magnitude of the diffusion resistance may have a considerable effect on the *form* of the photosynthetic rate curves, and unlike K' and k_4 that depend almost entirely on the nature of the plant's materials, it is open to a certain degree of experimental control. The consideration of d may, therefore, be carried rather further at the present time than that of either K' or k_4 . It is unfortunate that in the paper of Maskell's already quoted there is a slip in the treatment of d ($=D$ in Maskell's notation) that results in the statement that "the corner of the CO_2 curve will be sharp for relatively high values of D (the resistance to diffusion of CO_2 into the cell)...." This is equivalent to saying that increasing the resistance to diffusion increases its rate, and the error clearly arises from a misinterpretation of the differential equation given for the rate of increase of photosynthetic rate with increase of external carbon dioxide concentration (1928*b*, p. 522). The reverse statement is, in fact, true as reference to the curves of Fig. 2 will at once show. Other things being equal, the *lower* the value of d the sharper will be the inflection of the curve.

It is interesting at this point to compare the various workers' results having regard to the conditions of diffusion imposed by their experimental arrangements. Maskell, comparing the results of Harder with those of Blackman and Smith (Fig. 1) for the same species, concluded that the differences were due in the main to a lower diffusion resistance in Harder's experiments. Since Harder immersed his *Fontinalis* in carbon dioxide buffered bicarbonate solutions, and Blackman and Smith used a slow stream of carbon dioxide solution,

the former was probably dealing with the lower resistance as supposed. As mentioned above, however, this would according to theory lead to a sharper, not to a more gradual, inflection of the curves, and the two sets of results cannot be harmonised along the lines suggested. Differences of K' , k_4 , or P_T might produce the observed effect. The fact that the same species was used for both investigations appears at first sight to reduce the likelihood of large differences in any of these quantities, yet it is not impossible, the material having been collected in widely separated districts, that different "physiological strains" were involved. The experimental techniques differed, however, in many respects besides those involving the conditions of diffusion, and it is very doubtful whether the experiments fixing the points along the Blackman and Smith curve are strictly comparable. Later work with the same species (James, 1928) involving both bicarbonate and carbon dioxide solutions always yielded curves with gradual inflections. The use of bicarbonates to provide carbon dioxide solutions with low diffusion resistances is open to the objection that physiologically unbalanced sodium or potassium ions are introduced simultaneously, and that as assimilation proceeds hydroxyl ions accumulate in the immediate neighbourhood of the chloroplasts. Unless it can be shown that neither of these affects the photosynthetic mechanism, and also that the abundant HCO_3 ions cannot be assimilated as such, comparisons between the two classes of solutions must remain very uncertain. The following experiment (Fig. 4) performed with a Wilmott (1921) "bubbler" shows that depression of photosynthetic rate occurs immediately on immersing a spray of *Elodea* in a bicarbonate solution of moderate concentration. The absence of any response to stirring the solution shows that the depression is not due merely to the establishment of a diffusion gradient as in free carbon dioxide solutions. The results reported by Harder are, therefore, open to rather grave objections, even though there was no obvious injury to the plant during his experiments.

A safer rough comparison may be made between the results recently reported by Van den Honert (1930) using *Hormidium flaccidum* filaments, and those of the author for *Fontinalis*. Van den Honert passed a rapid gas stream over his alga, that was moistened only by a thin film of water. This and the structure of the organism ensured a resistance to diffusion, very much lower than that in the *Fontinalis* experiments where a stream of water was made to pass slowly over the plant. In accordance with theoretical expectation Van den Honert's curves show much the sharpest inflections. It is

also interesting to notice that the results for Stålfelt's pine needles (Fig. 1; from Lundegårdh, 1931) with their sunken stomata and heavy cuticles suggest a high resistance to diffusion.

Comparisons of this sort are at the best very uncertain. Direct comparisons were made by the author (1928) in experiments with *Fontinalis* in which the same material was used throughout, and all conditions were kept constant save the rate at which the pure carbon dioxide solution was passed over the plant. The effect of moving the solution instead of allowing it to remain stationary in contact with the plant, is to break down the carbon dioxide diffusion shells that would otherwise be established round it, and so to move the higher con-

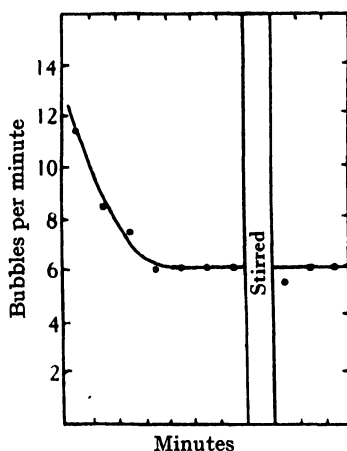


Fig. 4. Rate of bubble formation minute by minute by a shoot of *Elodea canadensis* in a 1 per cent. solution of sodium bicarbonate.

centrations nearer to the plant. Other things being equal, the faster the movement the shorter will be the diffusion path, and the lower the resistance in consequence. Fig. 5 gives the results of one such experiment, and by comparing it with Fig. 2 it will be seen at once that reducing the diffusion resistance has the general effect predicted by theory. In Fig. 5 the lines are not freehand curves, but are calculated from the deduced equation (5) (p. 26). Values of C_s and r were obtained from the experimental results, and converted into convenient units (gm. mols, minutes). K' , A ($=k_dLP_T$) and the two values of d that could not be measured directly were obtained by approximation. Results were also available that allowed the experimental error in the determinations of rate to be calculated by the usual statistical methods (Fisher, 1925) from which it appeared that only

values lying more than 0.058×10^{-6} units from the experimental values could be considered significantly different ($P=0.05$). The differences between experimental and theoretical rates were always well within these limits (Table II), and the theory may be considered to give a close numerical prediction of the facts.

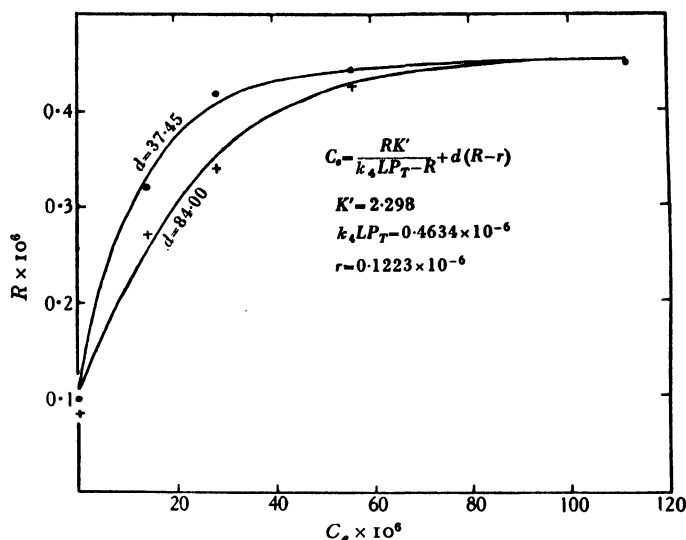


Fig. 5. Comparison of calculated rates of photosynthesis of *Fontinalis* with the experimental values for two different values of the diffusion resistance (d). Calculated values are represented by the continuous lines and experimental results by dots ($d=37.45$) and by crosses ($d=84.00$). Details of the calculation and meaning of symbols are given in the text.

TABLE II

Rate of photosynthesis by *Fontinalis antipyretica* at 20°C .

$$K' = 2.298; A (=k_4 I.P_T) = 0.4634 \times 10^{-6}; r = 0.1223 \times 10^{-6}$$

$C_e \times 10^6$	$d = 37.45$			$d = 84.00$		
	Found	Calculated	Difference	Found	Calculated	Difference
0	0.0978	0.1089	+0.0111	0.0864	0.1089	+0.0225
14.1	0.3219	0.3350	+0.0131	0.2706	0.2564	-0.0142
28.2	0.4173	0.4100	-0.0073	0.3390	0.3452	+0.0062
56.4	0.4425	0.4406	-0.0019	0.4254	0.4309	+0.0055
112.8	0.4483	0.4530	+0.0047	—	—	—

TEMPERATURE

The effect of temperature on the rate of biological reactions is usually very marked. On account of this obviousness and because temperature can be easily measured and controlled much work has been done on temperature coefficients, and there is a good selection of data available for photosynthesis. It was such an investigation

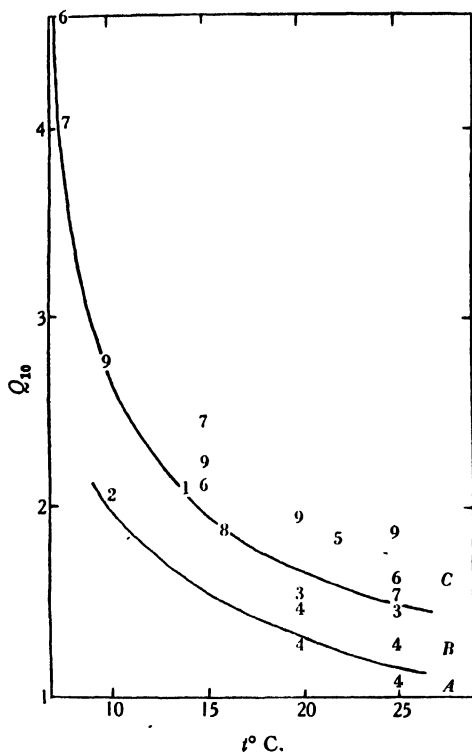


Fig. 6. Experimental values of Q_{10} obtained by various authors plotted against the middle temperature of the range employed. The numbers at the points indicate the authors and their plants according to the left-hand column of Table III. Sector *A* includes results from yellow leaves only; Sector *B* from green Angiosperm leaves; Sector *C* from green algae. Cf. Table III and text.

(Matthaei, 1904) that first emphasised the existence of serial reactions in the process. When compared with light, carbon dioxide, and other factor experiments, those on temperature suffer from a serious disadvantage, their comparative lack of discrimination. Carbon dioxide, as such, takes part in only one reaction, light may behave similarly, but it is quite clear that heat participates in many.

It is conventional to distinguish three types of temperature

relation, which can be summarised simply in terms of their corresponding temperature coefficients (Q_{10} = rate at $t + 10^\circ \text{C.}$ / rate at $t^\circ \text{C.}$). The three types are reactions independent of temperature, $Q_{10} = 1$; reactions with $Q_{10} = 1.2$ – 1.5 ; reactions with $Q_{10} = 2, 3$ or higher. Photochemical reactions are said to fall into the first class; physical processes, such as diffusion, into the second; and chemical reactions of the normal thermal type into the third. As a matter of fact the distinctions are far from rigid; exceptional photoreactions with coefficients as high as 3.2 have been recorded (see Griffith and McKeown, 1929), and the high values ascribed to thermal reactions fall off rapidly at high temperatures. There is, however, general agreement that the temperature coefficient of photosynthesis working at low light intensities approximates closely to 1 (e.g. Warburg, 1919, 1.06; Van den Honert, 1930, 0.9). We may, therefore, assume that the photoreaction involved is virtually independent of temperature. The situation is rather less obvious, however, when low temperatures and relatively high light intensities and concentrations of carbon dioxide are used. Experimental determinations of the temperature coefficient under these conditions have given the following values.

TABLE III

Plant	Temperature interval: $^\circ\text{C}$	Q_{10}	Investigator
(1) <i>Prunus lauro-cerasus</i>	9–19	2.1	Matthaei, 1904
(2) <i>Elodea canadensis</i>	7–13	2.05	Blackman and Smith, 1911 b
(3) <i>Sambucus</i> sp.	15–25	1.52	Willstätter and Stoll, 1918
	20–30	1.45	
(4) <i>Ulmus</i> sp. green leaves	15–25	1.48	Willstätter and Stoll, 1918
	20–30	1.29	
	15–25	1.30	
	20–30	1.07	
(5) <i>Ulva</i>	17–27	1.81	Osterhout and Haas, 1919
(6) <i>Chlorella pyrenoidosa</i>	5.4–10	4.3	Warburg, 1919
	10–20	2.1	
	20–30	1.6	
(7) <i>Chlorella</i> sp.	5–10	4.04	Yabusoe, 1924
	10–20	2.44	
	20–30	1.53	
(8) <i>Hormidium flaccidum</i>	12–20	1.87	Van den Honert, 1930
(9) <i>Hormidium flaccidum</i>	5–15	2.77	Van der Pauw, 1932
	10–20	2.24	
	15–25	1.96	
	20–30	1.87	
(10) <i>Chlorella vulgaris</i> (i)	4–14	4.6	Emerson, 1929 b
	8–17	2.85	
	16–26	1.5	
<i>Chlorella vulgaris</i> (ii)	4–15	4.58	
	9–18	3.40	
	15–25	2.43	

The temperature coefficients to be expected in the isolated reactions of our hypothetical sequence are as follows.

Stage	Q_{10}	Remarks
(1) Diffusion	1.2-1.3	Provided that the resistance offered to diffusion by cell wall and protoplast varies in the same way as that of water
(2) Formation of chlorophyll carbon dioxide compound	About 2-3	If uncatalysed; somewhat lower if catalysed by an enzyme
(3) Formation of chlorophyll formaldehyde peroxide	1	Direct determination is in agreement with results from the majority of photoreactions investigated
(4) Breakdown of chlorophyll formaldehyde peroxide	About 2-3	Somewhat lower if catalysed by an enzyme

There is nothing at first sight to decide between 2 and 4 as the controlling reaction when temperature is low, and any chance of progress along these lines depends largely on the existence of different and characteristic coefficients in the two reactions. Fig. 6, representing in graphical form the data of Table III, makes some interesting points apparent in spite of the fact that the figures were collected by different authors using different plants and methods. In the first place, there is a marked fall of the temperature coefficient with rising temperature, the rate of the fall being particularly rapid at the lower temperatures. The high values recorded by Warburg (1919) and Yabusoe (1924) are often regarded as conflicting with the lower values of Willstätter and Stoll (1918), but when all the available results are plotted together there is seen to be no good reason against regarding these as the two extremes of a continuous series. The lower line drawn on the graph separates the values obtained from yellow leaves (Sector *A*) from those of normal green varieties. The points lying between the two lines (Sector *B*) were all obtained from green Angiosperm leaves, while those falling above the upper line (Sector *C*) are all derived from green algae. Each of these sectors may be considered to represent a separate range of temperature coefficients characteristic of the assimilating agent concerned. Direct comparison of the green and yellow leaves (Sectors *A* and *B*) used by Willstätter and Stoll suggests that different temperature coefficients are associated with different contents of chlorophyll. The experiments of Emerson (1929*b*) on cultures of *Chlorella vulgaris* grown with different doses of iron and developing different amounts of chlorophyll do nothing either to confirm or destroy this idea. The results obtained were extremely irregular, the temperature coefficient sometimes rising and sometimes falling with increasing pigmentation.

Willstätter's results are most readily explained by supposing that in the yellow leaves the rate is almost at its maximum for the small amount of chlorophyll present even at low temperatures, and that an increase of temperature is, therefore, only able to produce a small effect. The true temperature coefficient of the reaction cannot appear under these conditions. In the green leaves with their excess of chlorophyll a higher temperature coefficient for a given temperature interval appears as expected. It is conceivable when chlorophyll is scarce as in the yellow leaves that the stage in which it is a reactant is governing the rate, but that when it is present in excess this reaction is able to become fast and the second thermal reaction, the breakdown of chlorophyll-formaldehyde peroxide under the influence of a peroxide then controls the rate. The affinity of chlorophyll for carbon dioxide is extremely high (Willstätter and Stoll, 1918), so when both chlorophyll and carbon dioxide are abundant it seems extremely unlikely that the first thermal reaction should be the slower of the two, and in all probability the temperature coefficients obtained are those of the second reaction. In this case the restriction of rate can obviously not be caused by a deficiency of peroxide, since this would be referable to one of the preceding reactions, and a more probable suggestion is that the concentration of the peroxide-splitting enzyme is here the retarding factor.

A further line of investigation is thus opened for normal green leaves. Reactions catalysed by oxidising and reducing enzymes in general appear to have temperature coefficients close to two, which show very little tendency to fall with rising temperature (Haldane, 1930). An exceptional case is that of a fat catalase (Nordefeldt, quoted from Haldane) with a $Q_{10} = 1.4$ over the range 0–20° C. This is in very marked contrast with the behaviour just described for the peroxidase involved in photosynthesis. Yabusoe (1924) examined the rate of production of oxygen from hydrogen peroxide by living *Chlorella* cells in the dark, and found that the temperature relations were identical with those of oxygen production by photosynthesis in brilliant light, i.e. there was a high but rapidly falling Q_{10} . We may conclude either that the peroxidase in question has unusual temperature relations for an oxidase, or that the enzyme *in situ* behaves differently from the enzyme when extracted. Van der Paauw found that his values remained remarkably constant at different times of the year, and when he used different strains of *Hormidium*. This seems to tell strongly against any idea that the variation with temperature is solely due to some obscure dependence on protoplasmic condition. Further in-

vestigations of the temperature coefficients of leaf peroxidase reactions might do much to elucidate the kinetic distinction between the thermal reactions of photosynthesis. It is, for example, conceivable that the difference between the coefficients of Sectors *B* and *C* depends on a difference between Angiosperm and algal oxidases, and it would be interesting to know whether this may be given preference over the possible alternatives.

Using the Arrhenius equation in the form

$$\log_e Q_{10} = R e \left(\frac{1}{T_1} - \frac{1}{T_2} \right),$$

the apparent energy of activation, *e*, can easily be calculated from the Q_{10} in calories per gm. mol. (*R*=gas constant: T_1 and T_2 , two temperatures, ten degrees apart, on the absolute scale). Thus Van der Paauw (1932) gives the energy of activation of heat-retarded photosynthesis between 15° and 25° C. as 11,400. This seems at first sight to be a more rational, or at least a more impressive, method of expressing the results. The advantage is, however, illusory. In the first place *e*, being strictly related to Q_{10} , is no more diagnostic of its reaction, and since the assumptions of the Arrhenius equation are not applicable with certainty to enzymic and living systems (see Haldane, 1930, p. 68) definite error may be introduced. Van der Paauw, it should be mentioned, comes to the same conclusion on somewhat different grounds, and points the finger of scorn at those who yield to the temptation offered. Emerson (1929*b*) gives values of this kind for assimilation in *Chlorella vulgaris*, but his results are so irregular when calculated back as Q_{10} 's that they must on this account also be received with reserve. (Cf. the very regular and repeatable values obtained by Van der Paauw with *Hormidium*.)

CONCLUSION

In the foregoing an attempt has been made to consider photosynthesis as a heterogeneous photochemical reaction, and to show that the known facts are open to quantitative explanation in these terms. It is not supposed that the very general equations developed are able to explain the details of the photosynthetic mechanism. Indeed it has been emphasised that more than one hypothesis concerning the nature of the individual reactions (and not the Willstätter theory alone) lead to the same results. This may be regarded either as a strength or a weakness of the method. Progress in the study of photosynthesis is, however, likely to be made if future

investigations are directed towards determining experimentally the nature, values and degrees of "constancy" of the terms involved in such velocity equations. It is suggested that this would be more profitable than continuing the hunt for the perfectly kinked curve.

REFERENCES

- ARNOLD, A. Der Verlauf der Assimilation von *Helodea canadensis* unter konstanten Aussenbedingungen. *Planta*, **13**, 529. 1931.
- BLACKMAN, F. F. Optima and limiting factors. *Ann. Bot.* **19**, 281. 1905.
- BLACKMAN, F. F. and MATTHAEI, G. L. C. Experimental researches on vegetable assimilation and respiration. IV. A quantitative study of carbon dioxide assimilation and leaf temperature in natural illumination. *Proc. Roy. Soc. B*, **76**, 402. 1905.
- BLACKMAN, F. F. and SMITH, A. M. Experimental researches on vegetable assimilation and respiration. VIII. A new method for estimating the gaseous exchanges of submerged plants. *Proc. Roy. Soc. B*, **83**, 374. 1911a.
- Experimental researches on vegetable assimilation and respiration. IX. On assimilation in submerged water plants and its relation to the concentration of carbon dioxide and other factors. *Proc. Roy. Soc. B*, **83**, 389. 1911b.
- BOYSEN-JENSEN, P. and MÜLLER, D. Die maximale Ausbeute und der tägliche Verlauf von Kohlensäureassimilation. *Jahrb. f. wiss. Bot.* **70**, 493. 1929.
- BRIGGS, G. E. Experimental researches on vegetable assimilation and respiration. XIII. The development of photosynthetic activity during germination. *Proc. Roy. Soc. B*, **91**, 249. 1920.
- Experimental researches on vegetable assimilation and respiration. XX. The energetic efficiency of photosynthesis in green plants; some new data and a discussion of the problems. *Proc. Roy. Soc. B*, **105**, 1. 1929.
- Experimental researches on vegetable assimilation and respiration. XXI. Induction phases in photosynthesis and their bearing on the mechanism of the process. *Proc. Roy. Soc. B*, **113**, 1. 1933.
- EMERSON, R. The relation between maximum rate of photosynthesis and concentration of chlorophyll. *Journ. Gen. Physiol.* **12**, 609. 1929a.
- Photosynthesis as a function of light intensity and of temperature with different concentrations of chlorophyll. *Journ. Gen. Physiol.* **12**, 623. 1929b.
- FISHER, R. A. *Statistical Methods for Research Workers*. Edinburgh. 1925.
- GRIFFITH, R. D. and McKEOWN, A. *Photoprocesses in Gaseous and Liquid Systems*. London. 1929.
- HALDANE, J. B. S. *Enzymes*. London. 1930.
- HARDER, R. Kritische Versuche zu Blackmans Theorie der "begrenzenden Faktoren" bei der Kohlensäureassimilation. *Jahrb. f. wiss. Bot.* **60**, 531. 1921.
- Über die Bedeutung von Lichtintensität und Wellenlänge für die Assimilation farbiger Algen. *Zeit. f. Bot.* **15**, 305. 1923.
- HINSHELWOOD, C. N. *The Kinetics of Chemical Change in Gaseous Systems*. 3rd ed. Oxford. 1933.
- JAMES, W. O. Experimental researches on vegetable assimilation and respiration. XIX. The effects of variations of carbon dioxide supply upon the rate of assimilation of submerged water plants. *Proc. Roy. Soc. B*, **103**, 1. 1928.
- KOSTYCHEV, S. Die Neue Vorstellung von der Photosynthese. *Planta*, **13**, 778. 1931.
- LUNDEGÅRDH, H. *Environment and Plant Development*. Trans. E. Ashby. London. 1931.
- MASKELL, E. J. *The Theory of Limiting Factors*. Thesis, Cambridge. 1925.

- MASKELL, E. J. Experimental researches on vegetable assimilation and respiration. XVII. The diurnal rhythm of assimilation in leaves of cherry laurel at "limiting" concentrations of carbon dioxide. *Proc. Roy. Soc. B*, **102**, 467. 1928a.
- Experimental researches on vegetable assimilation and respiration. XVIII. The relation between stomatal opening and assimilation. A critical study of assimilation rates and porometer rates in leaves of cherry laurel. *Proc. Roy. Soc. B*, **102**, 488. 1928b.
- MATTHAEI, G. L. C. Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxide assimilation. *Phil. Trans. Roy. Soc. B*, **197**, 47. 1904.
- MELLOR, J. W. *Chemical Statics and Dynamics*. London. 1904.
- MICHAELIS, L. and MENTEN, M. L. Die Kinetik der Invertinwirkung. *Biochem. Zeits.* **49**, 333. 1913.
- MONTFORT, C. and NEYDEL, K. Zur Beurteilung der "Inaktivierung" und des "Zeitfaktors" der Lichtwirkung bei der Assimilation stomata-freier Schatten-Farne. *Jahrb. f. wiss. Bot.* **68**, 801. 1928.
- NERNST, W. *Theoretical Chemistry*. Trans. L. W. Codd. London. 1923.
- OSTERHOUT, W. J. V. and HAAS, A. R. C. The temperature coefficient of photosynthesis. *Journ. Gen. Physiol.* **1**, 295. 1919.
- PINCUSSEN, L. *Photobiologie*. Leipzig. 1930.
- REINKE, J. Photometrische Untersuchungen über die Absorption des Lichtes in der Assimilationsorganen. *Bot. Zeitung*, **44**, 161. 1886.
- ROMELL, L. G. Ueber das Zusammenwirken der Produktionsfaktoren. Eine kritische Studie. *Jahrb. f. wiss. Bot.* **65**, 739. 1926.
- SCHROEDER, H. Die Kohlendioxydversorgung der Chloroplasten. *Flora*, **117**, 270. 1924.
- SENER, G. *Outlines of Physical Chemistry*. 16th ed. London. 1930.
- SMITH, A. M. Experimental researches on vegetable assimilation and respiration. XII. The temperature coefficient of photosynthesis. A reply to criticism. *Ann. Bot.* **33**, 517. 1919.
- STILES, W. *Photosynthesis. The Assimilation of Carbon by Green Plants*. London. 1925.
- VAN DEN HONERT, T. H. Carbon dioxide assimilation and limiting factors. *Recueil des Trav. Bot. Néerl.* **27**, 149. 1930.
- VAN DER PAAUW, F. The indirect action of external factors on photosynthesis. *Recueil des Trav. Bot. Néerl.* **29**, 497. 1932.
- WARBURG, O. Ueber die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. I. *Biochem. Zeits.* **100**, 230. 1919.
- Ueber die Geschwindigkeit der photochemischen Kohlensäurezersetzung in lebenden Zellen. II. *Biochem. Zeits.* **103**, 118. 1920.
- WARBURG, O. and NEGELEIN, E. Ueber den Energieumsatz bei der Kohlensäureassimilation. *Zeits. f. physik. Chemie*, **102**, 236. 1922.
- Ueber den Einfluss der Wellenlänge auf den Energieumsatz bei der Kohlensäureassimilation. *Zeits. f. physik. Chemie*, **106**, 191. 1923.
- WARBURG, O. and UYESUGI, T. Ueber die Blackmansche Reaktion. *Biochem. Zeits.* **146**, 486. 1924.
- WILLSTÄTTER, R. and STOLL, A. *Untersuchungen über Chlorophyll*. Berlin. 1913.
- *Untersuchungen über die Assimilation der Kohlensäure*. Berlin. 1918.
- WILMOTT, A. J. Experimental researches on vegetable assimilation and respiration. XIV. Assimilation by submerged plants in dilute solutions of bicarbonates and of acids: an improved bubble counting technique. *Proc. Roy. Soc. B*, **92**, 304. 1921.
- WURMSER, R. *Recherches sur l'assimilation chlorophyllienne*. Paris. 1921.
- YABUSOE, M. Ueber den Temperaturkoeffizienten der Kohlensäureassimilation. II. Mitteilung über die Blackmansche Reaktion. *Biochem. Zeits.* **152**, 498. 1924.

HETEROTHALLY AND THE SEED HABIT VERSUS HETEROSPORY

By R. B. THOMSON

Department of Botany, University of Toronto

SOME months ago there were two notes in *Nature* on "Heterospory and the Angiosperms," the first (May 28th, 1932) by Professor R. Ruggles Gates and the second, in reply to it by Dr D. H. Scott (June 11th, 1932). They were the outcome of an article by the writer in 1927 on "Evolution of the seed habit in plants(1)." In this article the orthodox view which assumes that the spores of seed plants differ in size, that they are *mega*- and *micro*-spores, was first brought into question. It was also pointed out that this view had apparently met with undisputed acceptance ever since Hofmeister's classic comparative researches of the middle of the last century, although Hofmeister himself did not make any reference to difference in size. His comparison is tersely expressed and refers to the distinctive feature of the seed habit, the enclosure of the female spore; "Der Embryosack der Coniferen lässt sich betrachten als eine Spore, welche von ihrem Sporangium umschlossen bleibt(2)." He did, however, draw attention to the similarity of the endosporal prothallial development in heterosporous vascular cryptogams and seed plants, though his cautious statement does not reveal any inference on his part that the spores of the seed plant differ in size. Whether or not Hofmeister thought they differed, the view that they did differ gained general acceptance, although no measurements were ever made to substantiate it.

When the first measurements were made in 1927 and these disclosed only minor variations in the size of the pollen and seed spores the writer concluded that with respect to size of spore the seed plants are homosporous. Since the Gymnosperms showed almost complete uniformity of size and it was in the Angiosperms that differences were found, it was further concluded that the seed plants are ancestrally homosporous. In connection with the greater extent of difference in the Angiosperms and the greater diversity—equal spore size, male larger, and female larger—it was suggested that "ecological" factors both external and internal might be involved, and that an investigation along these lines might give significant results.

Dr Gates agrees that such factors may be operative and suggests

how they may apply, especially to the conditions in *Oenothera rubricalyx*. This is the form in which in 1929 he had found that the size of the mother cells was "about $4930\mu^3$ for the 'megaspore' mother cell and $12,630\mu^3$ for the 'microspore' mother cell at the end of meiosis" and had concluded, from the reversed size of the mother cells, that the old view could not hold. In connection with this conclusion Dr Gates points out that it was arrived at independently of a knowledge of the work done by the writer in 1927. Dr Scott in his reply in support of the older view, takes exception to Dr Gates' basis of comparison and gives as his reason that "In the heterosporous Pteridophytes there may be little or no difference in the size of the respective mother cells up to the time of their division." Dr Scott then suggests as the proper basis "the relative volume of the mature and functional microspores and megaspores." This would mean, in so far as the writer can judge, that Dr Scott would make comparison of size after the spores had developed their endoprothallia or gametophytes to the sex-cell stage. He, however, does not make clear why he has selected the product of the spores as the proper basis rather than the producers—the mother cells of Gates. To select either stage is not in conformity with the usual procedure in estimating the size of spores, and substantial reasons would have to be advanced before either basis could be accepted. The writer, however, followed the usual practice and measured the spores, when they were true spores, i.e. in the uninucleate condition. Dr Gates apparently accepts this basis, although his own conclusion was drawn from the size of the mother cell. Dr Scott apparently does not approve of either mother cell or uninucleate stage, as he suggests another as the proper basis for the determination of heterosporosity. It appears to the writer that Dr Scott's basis is a measure of prothallium rather than of spore, and that the differences he would find should be referred to as heterothallic rather than heterosporic. This is a significant distinction when applied to the seed plants, based on the fact that although heterosporosity always entails heterothally the reverse is not necessarily the case. For example, *Equisetum* is homosporous but heterothallic with the female prothallia many times the bulk of the male. A similar condition is found in many leptosporangiate ferns. Dr Scott's idea, however, has a valuable suggestion in it—the possibility that difference in sex may be a significant factor. Although this may not reveal itself in difference in size of spore it may in difference in size of prothallia, and sex difference and heterothally are recognised characteristics of the seed habit.

There is another point which must be met if the spores of seed

plants are really to deserve the designation—*mega*- and *micro*-spore. As it concerns fossil plants Dr Scott's statement is pertinent: "As a palaeobotanist, I have no prejudice in favour of the origin of the flowering plants from heterosporous Cryptogams." I wish I could have said the same in 1904, but I was then and for many years afterwards a confirmed adherent of the orthodox view. That was the year when the brilliant work of Dr Scott in collaboration with Professor F. O. Oliver established the Pteridospermae. I had then in the press a paper on "The Megaspore Membrane in the Gymnosperms" (3) and wanted to see these ancient and primitive seed plants to find out if the spore coat was as thick in them as in the megaspore of heterosporous forms. I was courteously allowed to examine all the slides and carefully searched in them for a megaspore coat comparable in thickness to what I had found in true heterosporous forms such as *Sclaginella* and the leptosporangiate ferns, but was disappointed to find the spore coat not appreciably thicker than in the living Cycads. Here I had already been disconcerted to find it no thicker in the seed spore than in the pollen spore, although I knew that in true heterosporous forms the megaspore coat was several times as thick as that of the microspore. Whether or not I was right in thinking that I should find a very thick coat on the female spore of seed plants if they were heterosporous, it was my disappointment in not finding one that finally stimulated me to measure the size of the spores, and prove, to my own satisfaction at least, that the spores of seed plants do not differ in size.

There was no small amount of trouble entailed in getting the measurements at the proper stage. As is well known the seed spore (embryo-sac) increases rapidly on the formation of its endoprothallium, but it is not so well known that the pollen spore also increases considerably in size during the formation of its prothallium. Neither spore has a resting stage as a true spore, i.e. in the uninucleate condition. In this respect the spores of seed plants are very different from those of most free-sporing forms, especially where heterospory is involved. If the seed plants had had resting spores, with thick coats and storage products, they would then probably have been measured, as has been done in the case of spores of so many free-sporing forms, and their equality would never have been questioned¹.

¹ Several botanists, notably Professor Joseph Doyle, of Dublin, and Professor Lester W. Sharp, of Cornell, wish to see the idea that the seed plants are homosporous more generally accepted, and with this end in view have suggested that the spores in these plants be designated *andro*- and *gyno*-spore, instead of *pollen*- and *seed*-spore or *doulo*- and *angio*-spore. Needless to say I welcome any designation that will serve to eradicate the old idea that difference in size of spore is essential to the seed habit.

As a result of the recognition of the true condition of spore size in the seed plants the significant features of the seed habit stand out in bold relief and present a striking contrast to the heterosporous condition. The female spore in heterosporous forms, with its thick coat and abundance of stored food, represents a storage and resting stage, with no more food possible from the mother plant—patently an adaptation of the free-sporing habit; the female spore in the seed habit plants is without a thick coat or storage products and develops continuously from its uninucleate or true spore condition until it finally enters its resting and storage stage on the formation of reproductive cells or even later. In heterospory there is great difference in spore size and little in that of the sporangium; in the seed habit there is little difference in spore size but great difference not only in size but in elaboration of the sporangium. In a word, heterangy in combination with homospory and heterothally forms the distinctive features of the seed habit while heterospory represents the culmination of the free-sporing habit.

REFERENCES

- (1) THOMSON, R. B. Evolution of the seed habit in plants. *Trans. Roy. Soc. Canada*, **21**, 229-72. 1927.
- (2) HOFMEISTER, W. *Vergleichende Untersuchungen*. 1849-51.
- (3) THOMSON, R. B. The megaspore membrane in the Gymnosperms. *Univ. Toronto Studies, Biol. Series*, No. 4, pp. 3-64, Pls. 1-5. 1905.

The above paper came to us through the hands of Dr D. H. Scott, and was sent to press before the sudden announcement of his death.

THE EDITORS.

UEBER DIE GESTALT DER BÄUME

VON DR HEINRICH HÄRDTL

Tetschen, Czeckoslovakia

(Mit 6 Textfiguren)

Die Gestalt der Bäume ist allen wohlbekannt und in Sommer und Winter kann man auf Grund dieser die betreffende Art erkennen. Jede Baumart hat seine Eigenheit, aber doch vieles Gemeinsame. Dieses Gemeinsame, das Verhalten zu bestimmten integrierenden Ausseneinwirkungen, wie vornehmlich der Schwerkraft, will ich hier kurz zusammenfassen und versuchen, das Problem der Gestaltung höherer Pflanzen unter diesem Blickwinkel zu betrachten.

Ehe ich auf die grossen, verschieden zusammengesetzten Organe der Pflanze eingehe, soll einiges Prinzipielle an den Laubblättern besprochen werden.

Bei den Laubblättern konnte nachgewiesen werden (1-4), dass sie sich in der Horizontalebene im Gleichgewicht befinden. Ein Laubblatt hat—von Schiefblättern abgesehen—nur eine Symmetrieebene und diese liegt in der Mittelrippe. Zu beiden Seiten sind die Blattmassen gleichmässig verteilt. Stiel und Mittelrippe bilden, von oben betrachtet, eine Gerade. Diese Stellung besitzen die Blätter während ihres ganzen Lebens. Halbiert man die Spreite, so bewegt sich der restliche Teil in der Ansatzstelle am Stiel. Stiel und Mittelrippe liegen nun in keiner Geraden mehr, sondern bilden einen Winkel (Fig. 1). Bei allen gleichgestalteten Blattspreiten ist dieser Winkel gleich.

Von der Tatsache der Gleichartigkeit der Blattareale zu beiden Seiten der Mittelrippe ausgehend, war eine Störung der Stielbelastung angenommen worden. Unter dieser Voraussetzung musste eine Ergänzung des fehlenden Gewichtes die Bewegung hintanhaltend (Fig. 1). Tatsächlich blieb bei derartiger Belastung die Bewegung aus, aber auch eine vollzogene Bewegung konnte rückgängig gemacht werden.

Eine ungleiche Belastung wurde auch durch Anhängen von Gewichten ausserhalb der Mittelrippe erlangt. An grösseren Blättern wurde dies getan und man sah ebenfalls eine Bewegung der Spreite. Teilte man die Spreite entsprechend der Verlängerung des Stieles, ermittelte in jedem dieser Teile die Schwerpunkte und den senkrechten

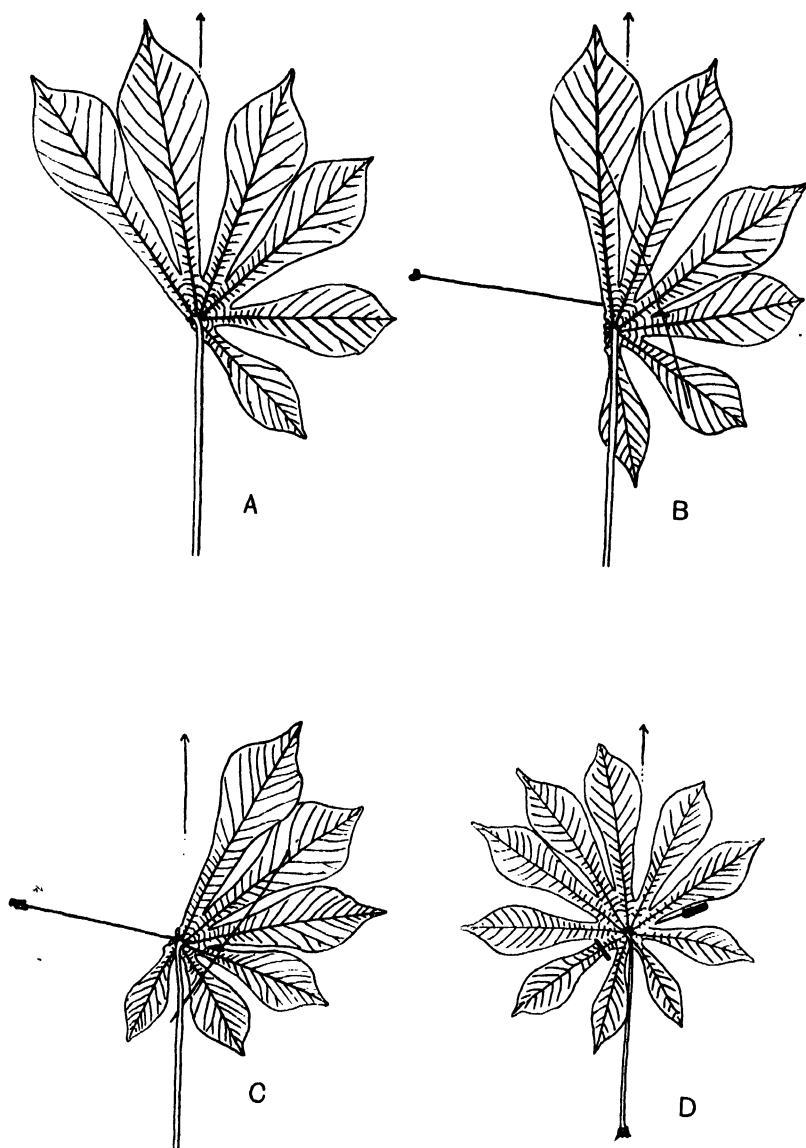


Fig. 1. Blätter von *Cecropia palmata*. 1/6 nat. Gr. Die Blätter wurden senkrecht von unten photographiert und dann gezeichnet. Der Pfeil kennzeichnet die Ausgangslage der Spreite. A, Die Spreite wurde annähernd halbiert. Die Gleichgewichtsbewegung beträgt 36 Grade. B, Das gleiche Blatt wurde belastet, indem an Aluminiumstäbchen ein Bleigewicht befestigt wurde. Die Ausgangsstellung wird annähernd zurückgewonnen. C, Eine Gewichtsvermehrung bewirkt eine Bewegung über die Ausgangslage hinaus. D, Ein anderes Blatt wurde nur ungleichseitig belastet und die Bewegung zur Herstellung des Gleichgewichtes beobachtet.

Abstand dieser zur Trennungslinie, so ergab sich nach der Formel $L_1 : L_2 = Q_1 : Q_2$ ein Gleichgewicht.

Diese Vorgänge wurden an einfachen und zusammengesetzten Blättern geprüft (1-4). Die Blätter vermögen eine Lageänderung jederzeit und auch im Dunkeln auszugleichen und eine gewichtssymmetrische Lage neuerdings einzunehmen. Diese Fähigkeit wird als Gleichgewichtsreiz oder Isoklinotropismus bezeichnet.

Das Licht ist nur dann von Einfluss auf die Lage des Blattes, wenn es unsymmetrisch auffällt: es kommt zu einer phototropen

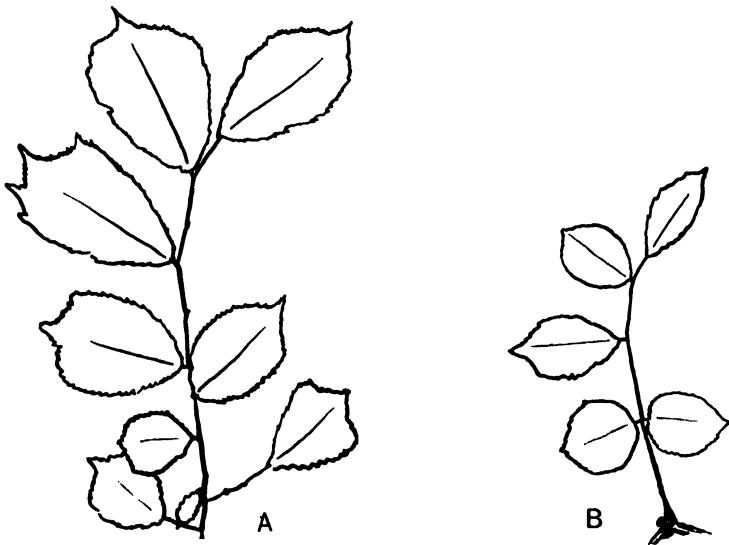


Fig. 2. A, Zweig von *Ulmus campestris*, an dem zwei Blätter entfernt worden waren.

B, Fiederblatt von *Sambucus nigra* in gleicher Weise behandelt. Man erkennt das gleiche Verhalten morphologisch verschiedener Organe. 1/4 nat. Gr.

Bewegung, durch die aber eine Gleichgewichtsstörung hervorgerufen wird. Dadurch entsteht ein Antagonismus, welcher eine Weiterleitung des phototropen Reizes bewirkt. Hier, weiter basal, kommt es ebenfalls zu einer Bewegung und ebenfalls zu einem Antagonismus, der wiederum eine Weiterleitung zur Folge hat. So sind die phototropen Bewegungen der Sprosssteile zu erklären. Somit können die Vorgänge im Blatt die Gestalt der ganzen Pflanze beeinflussen.

An Zweigen habe ich ebenfalls Versuche einer Gleichgewichtsstörung durchgeführt. Auch diese reagieren auf diesen Reiz und stellen sich in eine gewichtssymmetrische Lage (Fig. 2). Beobach-

tungen in der Natur lassen überall das gleiche Prinzip erkennen. Die Verzweigung ist vornehmlich an entlaubten Bäumen zu sehen und ich habe eingehend die Verzweigungsformen einheimischer Baumarten untersucht (5).

An den Aesten sieht man die gleichmässige Verzweigung oder bei Beschädigung Krümmung auch stärkerer Zweige zwecks gleichmässiger Lastverteilung (Fig. 3, 4 und 5). Die Ausbreitung der Zweige vollzieht sich nicht bei allen Pflanzen in einer Ebene wie bei Nadelhölzern, Linden oder Buchen, sondern bei manchen Baumarten bilden sie Stellungen nach verschiedenen Richtungen und das

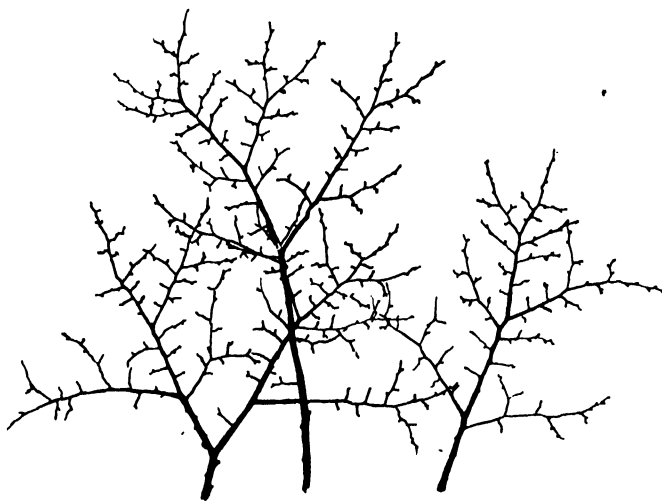


Fig. 3. Zweige einer Linde (*Tilia platyphyllos*)

Gepräge der Aeste bildet gleichsam eine Kombination aus verschiedenen mechanisch beanspruchten Teilen. Deshalb muss auch die Gleichgewichtsverteilung in der Vertikalebene im Hinblick auf die Gestaltbildung untersucht werden.

In der Vertikallage nehmen die Blätter eine bestimmte Stellung ein, jedoch wechselt diese im Laufe der Entwicklung im Gegensatz zu der in Horizontallage. Diese Profilstellung ist aber nicht nur von der Entwicklung, sondern auch von der natürlichen oder künstlichen mechanischen Inanspruchnahme abhängig: Je grösser die Belastung, umso geneigter stehen die Stiele und umso kleiner bleiben sie (6-8). Anatomisch entwickeln sie sich entsprechend der erhöhten mechanischen Inanspruchnahme im Sinne von Verbundbauten (11, 12).

Aber nicht nur die Stiele, welche direkt betroffen werden, verändern

sich, sondern auch die Spreiten werden bei diesen Versuchen in den Reaktionsverlauf hineingezogen: Je grösser die Belastung, umso mehr bleiben sie im Wachstum zurück. Da die Spreiten nicht in allen Teilen gleichmässig wachsen, werden natürlich die Teile am stärksten betroffen, welche am spätesten ihr Wachstum beenden und das ist meist das Breitenwachstum.

Mit der Grösse der Belastung ändert sich die Stellung zur Horizontalen. Je nach der Blattart kann bei bestimmter Belastung ein Maximum der Wachstumsbeeinträchtigung bei Stiel und Spreite eintreten.

Die Ursache dieser Wachstumsänderungen liegt wohl in der Stoffwechseltätigkeit. Es ist anzunehmen, dass, wie Resektionsversuche den Sitz von Wachstumsstoffen im Assimilationsgewebe

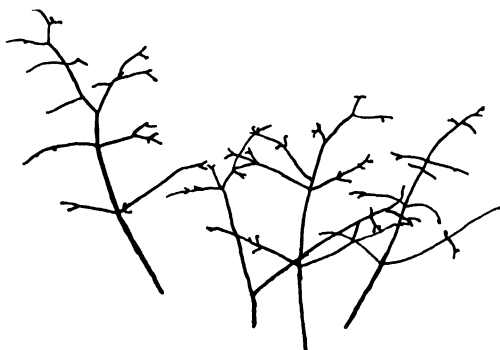


Fig. 4. Zweige eines Ahorns (*Acer pseudoplatanus*).

wahrscheinlich machen(8), Wuchsstoffe durch den geänderten Stoffwechsel in den Betriebsstoffwechsel einbezogen werden.

Versuche mit erhöhter mechanischer Inanspruchnahme ergaben an Zweigen einen entsprechenden Erfolg (Fig. 6). Belastungen zwingen die Zweige in eine schrägere Stellung, der Ast bleibt kleiner, beendet früher seine Entwicklung und darum werden auch nicht so viele Blätter oder Blattpaare angesetzt. Ausserdem erreichen die Blätter solcher belasteter Zweige nicht die normale Grösse und das Gesamtausmass der Blattflächen ist oft um die Hälfte kleiner.

Aus allen diesen Versuchen lässt sich erkennen, dass sich die Organe in vertikaler und horizontaler Ebene in einer Zwangslage befinden. Wenn man das jeder Pflanze eigene Verzweigungssystem in der Lot- und Wagrechten mit berücksichtigt, dann können wir leichter verstehen, warum die Bäume diese oder jene Kronenform besitzen,

im geschlossenen Verbande andere Stammbildung aufweisen als freistehend (9, 10), aber auch warum gewisse Bäume nicht so leicht über eine gewisse Höhe hinauswachsen können.

Die Grösse der Bäume scheint durch diesen Faktor bestimmt zu werden und es eröffnet sich die Möglichkeit, etwas über die Grösse der verschiedenen Baumarten zu sagen. Hat ein Baum eine sympodiale Verzweigung, so ist es meist so, dass vom Hauptstamm ein starker Seitenast abzweigt und dadurch der erstere etwas aus seiner Richtung gedrängt wird. Dadurch gelangt er aber in eine zur Schwere Wirkung andere Lage. Je stärker diese Abdrängung ist, umso geringer



Fig. 5. Zweige einer Ulme (*Ulmus campestris*).

wird sein Wachstum sein und er begrenzt damit selbst seine Grössenentwicklung.

Von den Ergebnissen dieser Belastungsversuche aus wird verständlich, warum Baumkronen meist ein elliptisches, bzw. kegelförmiges Profil besitzen und Bäume mit mehrfacher Abzweigung von gleicher Stelle kein besonderes Höhenwachstum aufweisen gegenüber Bäumen mit gut entwickeltem und durchgehendem Hauptstamm.

Diese Ergebnisse über die Abhängigkeit der Gestaltung der Bäume von der eigenen oder hinzugefügten Last gewinnt für obstbauliche Fragen einige Bedeutung. Die Fruchtlast würde vermutlich ein noch vorhandenes Wachstum an Zweigen einschränken. Es ergibt sich ein weiterer Gesichtspunkt für die Bedeutung des Ausästens, der Unterstützung der Zweige u.s.w., denn durch Entlastung wird nicht nur ein bestehendes Wachstum, wie Knospenentwicklung, unbehinderter sein, sondern auch der Betriebsstoffwechsel weniger angespannt und

dadurch eine Entlastung des Baumes in ihren Stoffumsätzen bedingt sein. All dieses kann nicht nur günstige Folgen für die diesjährige Fruchtentwicklung und Reifung, sondern auch für den nächstjährigen Fruchtansatz mit sich bringen.

Rein theoretisch schiene mir bemerkenswert, dass eine Entwicklung der Pflanzen von niederen zu höher organisierten Formen nur



Fig. 6. Zweige von *Syringa vulgaris*. Ein Zweig wurde mit 20 g. belastet und zwar wurde nach einiger Zeit der Entwicklung dieses Gewicht weiter apikalwärts angehängt, um die Lastwirkung zu erhöhen. *B* = Belastung. Man sieht kleinere Blätter und kürzere Internodien. Das Wachstum ist nicht vollkommen abgeschlossen.

durch Ueberwindung der mechanischen Inanspruchnahme mit Hilfe entsprechender baumechanischer Ausgestaltung möglich wurde. Die allmähliche Grössenentwicklung lässt sich erdgeschichtlich nachweisen (11,12) und die höchstgebauten Pflanzen in den letzten Erdperioden erkennen. Dieses Ergebnis kann durch die hier dargelegte Abhängigkeit der Gestaltung von den eigenen Lastwirkungen bestätigt werden.

LITERATUR

- (1) HÄRDTL, H. (1925). Beitrag zur Erklärung der Blattlage am Spross. *Lotos*, **73**, 219–20. Prag.
- (2) — (1927). Licht und Schwerkraft in ihrer Wirkung auf die Stellung des Blattes. *Beirr. Biol. Pfl.* **15**, 275–326.
- (3) — (1933). Beitrag zur Kenntnis der Gleichgewichtsbewegung an Blättern unter besonderer Berücksichtigung der Bewegungsformen und des Wassergehaltes in den Tragorganen der Spreiten. *Bot. Archiv*, **35**, 251–306.
- (4) PRINGSHEIM, E. G. (1931). Lageveränderungen an Blättern nach Symmetriestörungen. *Flora*, N.F., **26**, 61–110.
- (5) HÄRDTL, H. (1934). Beitrag zum Problem der Gestaltung höherer Pflanzen unter dem Einfluss der Schwerkraft. (Eine obstbauliche Vorstudie.) *Gartenbauwiss.* (in the press).
- (6) — (1927). Die Wirkung mechanischer Inanspruchnahme auf Bau und Biegefestigkeit der Blattstiele. *Bot. Archiv*, **18**, 61–92.
- (7) — (1932). Ueber die Wirkung mechanischer Inanspruchnahme durch Belastung bei Laubblättern. *Bot. Archiv*, **34**, 81–101.
- (8) — (1934). Untersuchungen an Laubblättern über die Beziehungen zwischen Stiel und Spreite bei verschiedener Belastung und Resektion. *Beihefte Bot. Centralbl.* (in the press).
- (9) KLEIN, L. (1926). *Forstbotanik. Handbuch der Forstwissenschaften*, hrsg. von H. Weber, **1**, 635–887. Tübingen.
- (10) LUNDEGÅRDH, H. (1916). Physiologische Studien über die Baumarchitektonik. *Kunigl. Vet. Akad. Handl.* **56**, 1–64.
- (11) RASDORSKY, WL. (1928). Das baumechanische Modell der Pflanze. *Ber. Dtsch. Bot. Ges.* **46**, 48–104.
- (12) ZIMMERMANN, W. (1930). Der Baum in seinem phylogenetischen Werden. *Ber. Dtsch. Bot. Ges.* **48**, 34–49.

VARIATIONS IN THE MEDULLARY BUNDLES OF *ACHYRANTHES ASPERA* L. AND THE ORIGINAL HOME OF THE SPECIES

By A. C. JOSHI

Department of Botany, Benares Hindu University, India

(With 2 figures in the text)

THE stem of a number of species of *Achyranthes* (*A. aspera* L.¹, *A. crispa*², *A. argentea* Lamk.² and *A. bidentata* Blume³) has been shown to possess a pair of collateral medullary bundles inside the normal primary vascular ring. These bundles in an internode lie opposite to each other, in a line with the pair of leaves just below, and in most of the species, namely, *A. bidentata*, *A. argentea* and *A. crispa*, they run in the same lines throughout the length of an internode, quite freely and without touching each other. In every adjacent internode in these species, as in *A. aspera* also, they change their position to a different plane perpendicular to their first position. The disposition of the two medullary bundles in the internodes of *A. aspera*, however, varies a great deal in material collected from different places, though it agrees quite exactly with the other species in the ground plan. These variations further have been found to show a very regular sequence, and as they seem to throw some light on the original home of the species, it has been thought worth while to report the following observations.

The material used in the investigation has come from the following four places:

Place	Longitude	Latitude
1. Lahore	74°26' E.	31°27' N.
2. Bombay	72°54' E.	18°55' N.
3. Calcutta	88°30' E.	22°30' N.
4. Benares	83°00' E.	25°15' N.

The material of Bombay plants was sent to the writer by Professor R. H. Dastur. It was preserved in formalin and was examined by cutting hand sections. The material from other places was examined by the writer on the spot in the fresh condition by cutting it with a safety razor blade. This latter method is possible owing to the fact that the medullary bundles can be quite clearly seen even on cutting

¹ Dastur(1) and Joshi(2).

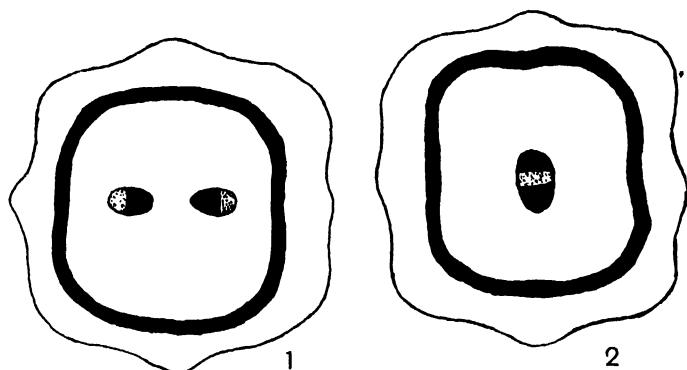
² Schmid(3).

³ This has been seen by the writer though the observations are not yet published.

an internode with a sharp knife. They appear as green structures in the white pith, and in this manner it is possible to examine a large number of plants completely within a short time. A microscope is needed only when the detailed structure of the bundles is to be seen.

OBSERVATIONS

(a) *Lahore plants*. The structure of the Lahore plants has been fully described by the writer elsewhere (2), and here it will be sufficient to give just a brief summary of the results obtained. The two free medullary bundles (Fig. 1), such as were described by Dastur (1) to be



Figs. 1 and 2. *Achyranthes aspera*. Transverse sections of the stem: 1, just below a node, showing two free medullary bundles; 2, through the middle of an internode, showing the medullary bundles fused and forming one amphixylic bundle. The outer normal ring of bundles is shown in black. In the medullary bundles xylem is shown in black and phloem by dots. There is always a cambium in between the xylem and the phloem but this is not represented. Magnified about 20 times.

of universal occurrence in all the internodes of the Bombay plants, are found only in a few internodes in Lahore plants. They are mostly restricted to only two or three internodes of the stem (both of the main stem and the branches) just below the various flowering spikes. In every plant examined—and no less than 50 were studied completely—it was found that, in an overwhelming majority of the internodes, the two medullary bundles are free only for a short distance just above or below a node. Through their greater length, they are found to run together and unite with each other to form a single strand in the centre of the pith which could be described either as a double bundle or as a single bundle of an amphixylic type (Fig. 2). The various changes that lead to the formation of this type of bundle are also described in the paper mentioned above.

(b) *Bombay plants.* The material of Bombay plants sent by Professor R. H. Dastur consisted of 49 pieces of stem, each varying in length from 4 to 6 in. These provided 124 internodes. In 40 pieces, comprising 94 internodes, the medullary bundles were found to remain perfectly free from each other throughout their length. In nine pieces alone out of 49, amphixylic-fused medullary bundles were seen. These nine pieces comprised 30 internodes, and out of these 22 showed the above condition. In this way out of a total of 124 internodes examined, in 102 free medullary bundles were found, while in less than 20 per cent., in 22 only, these bundles were found to run through a smaller or longer length of the internode in the fused condition. From their thickness, hardness, amount of secondary growth, and in three cases from the presence of the root, it was concluded that the pieces showing fused bundles had come from near the base of the plant.

Thus in Bombay plants, the fused medullary bundles are not so conspicuous as in the Punjab plants. In fact Dastur(1), who was the first person to describe the medullary bundles of *Achyranthes aspera* and whose material also came from Bombay Presidency, failed to observe this condition. It may be then that in some plants this condition may be altogether absent. This is very probable from what I have seen of Calcutta and Benares plants. The fair representation of the fused condition in the material that I received from the same place may just be by chance. Even in this, however, the condition is of no such regular occurrence as in the Punjab plants. Besides being confined only to the base of the plant, it was often seen that the medullary bundles while they were fused in an upper internode were free in one just below. In the Punjab plants it is never so. The free medullary bundles are found only in a few internodes just below the inflorescence, and then lower down in all the internodes without any irregularity the fused condition is seen.

(c) *Calcutta plants.* About 25 plants were examined by the writer from the side of a road in the Royal Botanic Gardens, Sibpur. In all of these, with the exception of one, the medullary bundles were found to remain free in all the internodes of the plant. In the one plant in which conditions were different, it was only in one internode that the fused medullary bundles were seen. This internode came from near the base of the plant.

(d) *Benares plants.* At Benares about 50 complete plants were studied. These were taken from every type of situation where these plants grow. No differences connected with situation were found,

but in taking all the plants together it was noticed that in about half of them, as in the great majority of Calcutta plants, the medullary bundles remained quite free in all the internodes of the plant. In the other half, fused amphixylic bundles were seen here and there, as in the Punjab plants, but their occurrence was found to be very irregular. In one branch the sixth internode, in another the fourth, and in another the fifth internode from the inflorescence were found to show the fused condition of the medullary bundles, but all the internodes below these were found to possess two quite free medullary bundles throughout their length. In another branch three internodes consecutively, the third, fourth and fifth, showed the fused condition of the medullary bundles, but the internode below the fifth one, i.e. the sixth internode below the inflorescence, was found to show the free condition of the medullary bundles throughout its length. In another branch, the fourth and the sixth internodes below the inflorescence were found to show the fused condition of the medullary bundles, while the first, second, third and fifth internodes showed free bundles. In still another branch, it was the seventh internode below the inflorescence which showed the fusion of the medullary bundles. In this manner every branch was seen to possess a different condition from the others and the variations in the different branches exhibited no order, so the Benares plants in the structure of their medullary bundles are intermediate between the Calcutta and the Punjab plants.

DISCUSSION

From the presence of free collateral medullary bundles in the stem of all the investigated species of *Achyranthes* except *A. aspera*, preponderance of this condition in the Calcutta and Bombay plants of *A. aspera* and its presence even in the Punjab plants of the latter species (where the fused bundles are best seen) in a few internodes below every inflorescence and the highly specialised character of the fused bundles, it is quite clear that the occurrence of two free medullary bundles throughout the length of an internode is a primitive condition, and their fusion in the middle of an internode to form a single strand is a later and a derived condition. From this it follows that the Calcutta and Bombay plants, where the fusion of the medullary bundles is either completely absent or occurs only rarely and irregularly, are primitive, and the Punjab plants where the fused condition of the medullary bundles is dominant are more recent. Naturally, therefore, the first plants are to be regarded as near the

original home of the species, and the Punjab plants to be away from the original home. This would mean that in India the original home of the species was in the south in the tropics, and from there it has spread out northwards towards the sub-tropical parts.

The structure of Benares plants fully supports such a conclusion. This is a station intermediate in situation between Calcutta and Punjab, and the plants growing here also show a structure intermediate between that of Calcutta and Punjab plants.

The fact that the family Amaranaceae is mostly tropical in distribution also conforms with this hypothesis.

The thanks of the writer are due to Professor R. H. Dastur of Bombay University for material of Bombay plants.

REFERENCES

- (1) DASTUR, R. H. The origin and the course of the vascular bundles in *Achyranthes aspera* L. *Ann. Bot.* xxxix, 539-45. 1925.
- (2) JOSHI, A. C. Contributions to the anatomy of Chenopodiaceæ and Amaranaceæ, II. Primary vascular system of *Achyranthes aspera* L., *Cyathula prostrata* Blume and *Pupalia lappacea* Juss. *Journ. Ind. Bot. Soc.* x, 265-92. 1931.
- (3) SCHMID, W. Untersuchungen über den Bau der Wurzel und der Sprossachse der Amaranaceae. *Mitteilungen aus dem Bot. Museum der Universität Zürich*, cxxvii, 217-97. 1928.

SECONDARY (CHROMOSOME) ASSOCIATIONS IN UMBELLIFERAE AND BICORNES

By J. H. WANSCHER

Copenhagen

(With 6 figures in the text)

WHEN tracing the primary basic number of a family, association in groups of the bivalents in the metaphase plates may often give the clue. This grouping takes place just after or during the short prometaphase when the gemini after diakinesis come together into the centre of the cell. When the chromosomes arrange themselves in the equatorial plane, gemini (primarily paired chromosomes) of the same constitution, shape and size often stick together in groups of two, three or more. The number and size of these secondary groups is characteristic of the given material, and the arrangement formula may often give an indication of the numerical evolution.

In the following, secondary associations found in Umbelliferae and Bicornes are described and discussed.

I am indebted to Dr O. Hagerup for excellent cytological material of the Bicornes, which he has very kindly put at my disposal for investigation.

BICORNES

Hagerup (1927, 1928) reports the following chromosome numbers for the different families in the order Bicornes: Pyrolaceae, 23; Rhodoraceae, (6)¹, 12, 13, 24; Empetraceae, 13, 26; Ericaceae, 8, 12, 13, 18, 24, 26, 48; Vacciniaceae, 12, 24, 36, 30; Epacridaceae, 13; Clethraceae, 8, 16; Diapensiaceae, 6 (one species). From these numbers Hagerup deduces a 6-series (composed by numbers from different families), a 13-series and an 8-series. Secondary associations on the other hand seem to point very strongly to 4 as the primary basic number of the family.

The most common numbers in the order are 12 and its derivative 13. The series 8-12 points to a primitive basic number 4, in agreement with the results of the investigation of the secondary association of

¹ Hagerup reports 6 for *Phyllodoce coerulea*, but both in his drawings and in his slides I have counted 12. The low number given may be due to the common grouping of the chromosomes.

the metaphase chromosomes made upon Hagerup's very fine slides. *Empetrum nigrum* gave especially good material for the statistical definition of the composition of the groups of chromosomes. In nineteen clear polar views of the first metaphase I was able to count the chromosomes in each group, and the results are given in Table I. In the table are given the numbers of chromosomes within the groups

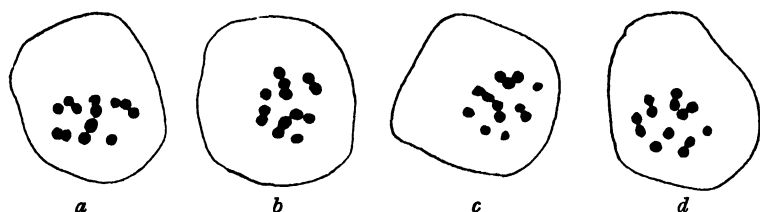


Fig. 1. Secondary associations in *Empetrum nigrum*, $n = 13$.
Maximum association 3-3-3-4.

indicated by IV to I, and the numbers of groups found in each cell. Fig. 1a-d are drawings of the four cells, nos. 1-4 in the Table.

Table I

Secondary associations in Empetrum nigrum ♂

Cell	IV	III	II	I	Groups
1 (Fig. 1a)	I	2	I	I	5
2 (Fig. 1b)	I	I	2	2	6
3 (Fig. 1c)	I	I	I	4	7
4 (Fig. 1d)	—	—	5	3	8
or	1	—	3	3	7
5	—	2	—	7	9
6	—	—	3	7	10
7	—	—	5	3	8
8	—	—	5	3	8
9	—	I	2	6	9
10	—	2	I	3	6
11	—	2	2	3	7
12	—	I	3	4	8
13	I	2	I	I	5
14	I	2	I	I	5
15	—	2	3	I	6
16	—	I	4	2	7
17	I	—	3	3	7
18	—	I	3	4	8
19	—	3	I	2	6
Ideal association					
	I	3	—	—	4
Maximum in each class:					
Found	I	3	5	7	10
Calculated	I	4	5	13	13

As it appears from the table there seems to be a maximum association of one group of four, three groups of three and five groups

of two bivalents. If we give the assumed primitive set of four chromosomes the letters *A*, *B*, *C* and *D*, we must repeat three of these three times and one of them four times to get 13, the haploid number of *Empetrum*.

$$\begin{array}{cccc} A_1 & A_2 & A_3 & A_4 \\ B_1 & B_2 & B_3 & \\ C_1 & C_2 & C_3 & \\ D_1 & D_2 & D_3 & \end{array}$$

The diploid plant contains two of each. These two come together in the prophase and form normal gemini, represented in the above formula by the letters A_1 , B_1 , C_1 , etc. Assuming that chromosomes with the same formula, A_1 and A_1 , etc., are able to pair primarily and that related pairs are able to come together in groups secondarily, we may find in a maximum grouping five pairs—two *A*, one *B*, one *C*, and one *D* pairs, and four groups of three—*A*, *B*, *C* and *D* groups;

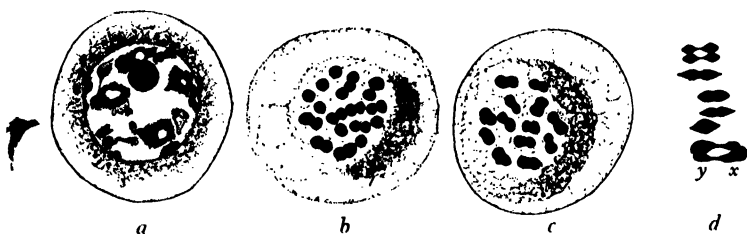


Fig. 2. *Empetrum hermaphroditum*, $n=26$. *a*, diakinesis; *b*, first metaphase in polar view; *c*, the same with thirteen double gemini; *d*, fragment of a nuclear plate, side view; note that the paired chromosomes are alike. 2500/1. By kind permission from O. Hagerup (1927).

and one group with four bivalents, four *A*'s. The results obtained are very near the calculated values. The classes IV and II are here of special importance. The maximum association, 4-3-3-3, is very nearly obtained in cell No. 1, which shows 4-3-3-2-1 (see Fig. 5*a-d*). The nineteen cells are from one pollen-sac.

In the tetraploid species *Empetrum hermaphroditum* (Lge.) Hagerup (1927), the secondary associations are also distinct. Hagerup's drawings, by kind permission given in Fig. 2, show in *c* thirteen pairs of bivalent chromosomes ($n=26$) and in *b* one IV, two III, five II and six I. The association, in groups bigger than two, points to a lower basic number than 13.

Erica cinerea, $n=12$, showed secondary association. Only two cells were numerically clear, owing to the too heavily stained plasma. The results were: (*a*) one III, three II and three I; (*b*) one III, two II

and five I (see Fig. 3). The trivalent group found in both cells points to the 4-series too, the probable maximum being 3-3-3-3.

Ledum groenlandicum, $n=13$, gave the following results in five cells: IV to I respectively: (1) 1, 1, 1, 4; (2) 1, 3, 0, 0; (3) 0, 4, 0, 1; (4) 1, 1, 3, 0; (5) 0, 3, 3, 1. In some of the cells there was a tendency in the groups and single chromosomes to fuse together more closely than in *Empetrum*. The groups were not spread so evenly as in this plant. This fusing may be due to the fixative (see Fig. 4).

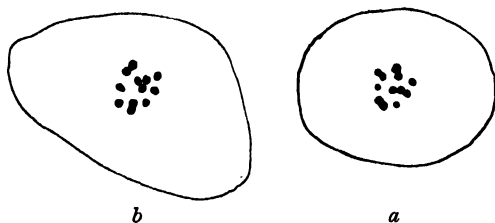


Fig. 3.

Fig. 3. *Erica cinerea*, $n=12$. Note the groups of three chromosomes.



Fig. 4.

Fig. 4. *Ledum groenlandicum*, $n=13$. Note the groups of three.

Phyllodoce coerulea, $n=12$ (see above). Association of the chromosomes is common but difficult to record numerically. Groups of two and three were found. Five was the smallest number of groups found.

In other, mostly tetraploid and hexaploid species of different genera, secondary pairing has been observed. Hagerup (1928) describes a similar phenomenon in *Arctostaphylos uva-ursi*, $n=26$, and finds that the chromosomes in the metaphase cohere in pairs, while in the anaphase they are entirely separated (see his Figs. 44 and 45 and also 10, 27 and 31). In none of the species have primary multiple associations been observed.

In short, four may be advanced as the probable primitive basic number of Bicornes instead of 6.

UMBELLIFERAE

In Umbelliferae the chromosome numbers vary from 6 to 48 haploid. This variation is illustrated in Table II (p. 62) taken from Wanscher (1933).

In the two first subfamilies, 8 is probably the basic number, but in the third 11 is the most common, being found in sixty-nine species. Probably 11 is of later origin than 8, both for the reason that 8 is found in most of the primitive genera investigated and for the reason

of secondary pairing in a species with the haploid number 22, pointing to a lower primary origin of this number than 11 from which it, the species being tetraploid, has arisen secondarily by simple reduplication. Secondary associations pointing to 8 are also found in some species with 24 chromosome haploid.

TABLE II

The variation of the chromosome numbers in the three subfamilies and the closely related Scandix (6a), Caulalis (6b) and Daucus (12) groups

Class	6	7	8	9	10	11	12	13	14	15	16	17
Hydrocotyloideae	—	—	4	—	—	2	—	—	—	—	—	—
Saniculoideae	—	2	7	—	—	—	—	—	—	—	—	—
Apiodeae	1	2	11	6	6	69	—	—	—	—	1	—
Umbelliferae	1	4	22	10	6	71	—	—	—	—	1	—
Genera 6a, 6b, 12	1	1	6	4	3	8	—	—	—	—	1	—

Class	18	19	20	21	22	23	24	32	33	42	48
Hydrocotyloideae	—	—	—	—	1	—	3	—	—	—	1
Saniculoideae	—	—	—	—	—	—	4	—	—	1	1
Apiodeae	1	—	—	—	3	—	—	1	1	—	—
Umbelliferae	1	—	—	—	4	—	7	1	1	1	2
Genera 6a, 6b, 12	—	—	—	—	1	—	—	—	—	—	—

Molopospermum cicutarium, with haploid 22, gave the best material, but the fixation was not perfect and it was often difficult to distinguish the single chromosomes in the groups.

The diakinesis was normal with equidistant gemini; in one cell only I found multivalent associations as expected in this tetraploid species. In Fig. 5 are shown eighteen bivalents and two quadri-valents in all 44 chromosomes. Root-tip counts confirm this number. In the following metaphases the chromosomes are mostly found in multivalent associations, far more frequently than the regular diakinesis indicates, hence this association must be of secondary origin; the equidistance of the groups tells us that the fixative has no influence on this grouping (Fig. 5b-e, groups of two and three bivalents in the first metaphase). In the anaphase the chromosomes are usually more spread out, as shown in Fig. 5f and g, two anaphase plates from the same cell drawn separately. In the second metaphase the grouping was found as distinct as in the first; Fig. 5h-q show different plates from this metaphase. The following table gives the different frequencies of the secondary groups.

According to the table the group of four is rare, being found in two cells only. The group of three, which in a plant with 22 chromosomes indicates a lower base number than $22/2$, is more common in

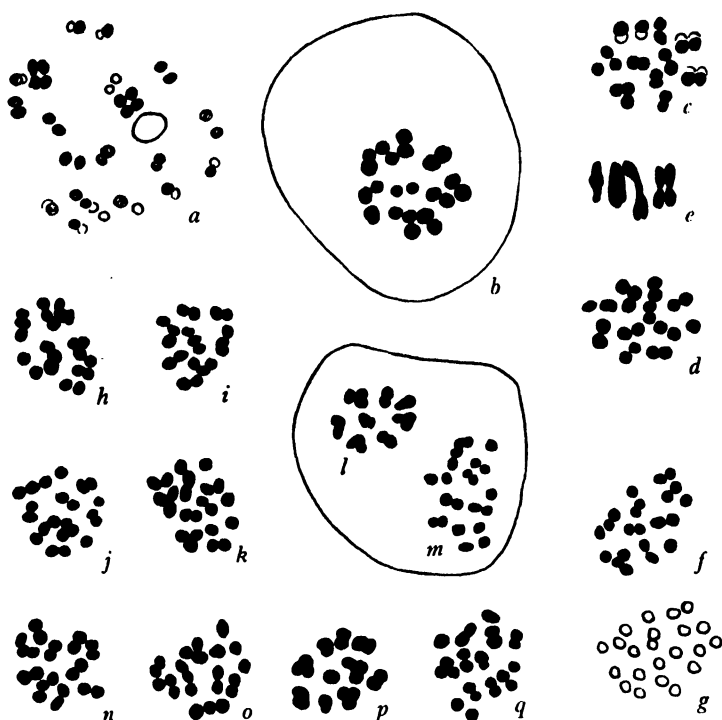


Fig. 5. Secondary association in *Molopospermum cicutarium*, $n=22$. *a*, diakinesis with two quadrivalent primary (?) associations; *b-d*, first metaphase in polar view; *e*, fragment of a metaphase plate in side view; *f, g*, first anaphase, the two plates drawn separately; *h-q*, second metaphase plates.

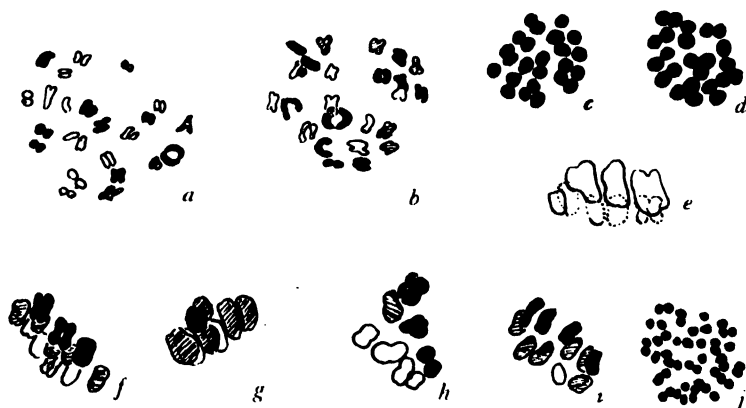


Fig. 6. *Hydrocotyle novae zelandicae*, *a-i*, $n=24$. *Hydrocotyle vulgaris*, *j*, $n=48$. First metaphase. *a, b*, diakinesis; *c, d*, first metaphase in polar view; *e-i*, the same in side view. The chromosomes lie together in groups.

TABLE III

Secondary associations in Molopospermum cicutarium

	Phase	IV	III	II	I	Total groups	Total chromo- somes
Fig. 5b	I metaphase	—	2?	7	—	9	22
c	"	—	1	8	3	12	22
d	"	—	1	8	3	12	22
f	I anaphase	—	2	6	4	12	22
h	II metaphase	1	4	3	—	8	22
i	"	—	4	4	—	8	20
j	"	—	3	5	3	11	22
k	"	—	3	5	4	12	23
l	"	—	4?	4	—	8	20?
m	"	1	—	6	6	13	22
n	"	—	2	8	—	10	22
o	"	—	4	3	4	11	22
p	"	—	3	5	2	10	21
q	"	—	1	8	5	14	24

some of the cells than one would predict on the basic number 8, i.e. $11 - 8 = 3$ groups of three. The group of two is very common, as expected in a tetraploid plant. The lowest number of groups found is eight, and this number leads us to the following formula:

$$4A-4B-4C-2D-2E-2F-2G-2H.$$

Assuming that only more or less homologous gemini group together secondarily, this formula allows a maximum association of eleven pairs of gemini, three groups of three or three groups of four. Of the few cells investigated ten out of fourteen favour this formula, three are against it and one uncertain. None of them favour the basic number 11 as they contain groups of either three or four chromosomes. The chromosome number of the second metaphase plates varies from 20 to 23 and indicates an occasional irregular first division.

In *Hydrocotyle* also "clumping" of the chromosomes was common. Fig. 6 illustrates this in *Hydrocotyle novae zelandicae* and *vulgaris*, the former in the drawings *a-i* and the latter in *j*. The former has haploid 24, and *a* and *b* show the diakinesis with normally distributed gemini; *c* and *d* show first metaphase in polar view, groups of two and three indicating a lower base number than $24/2$, probably 8 as in *Eryngium*, which shows the haploid numbers 8-24-48 in different species. The gemini lay rather closely in the groups, and in side view it was often impossible to count more than 8-11 "chromosomes" (see Fig. 6*e-i*). In Fig. 6*f* the members of the groups are distinguishable. Fig. 6*j* shows the first metaphase of *Hydrocotyle vulgaris*. The forty-eight bivalents lie also in groups of two and three chromosomes.

The question of the basic numbers in Umbelliferae and other families has been discussed elsewhere (see Wanscher, 1933) and will be referred to further in a later article in this *Journal*.

REFERENCES

- HAGERUP, O. *Empetrum hermaphroditum* (Lge.) Hagerup. A new tetraploid species. *Dansk Botanisk Arkiv*, 5, No. 2. 1927.
—— Morphological and cytological studies of Bicornes. *Dansk Botanisk Arkiv*, 6, No. 1. 1928.
WANSCHER, J. H. Studies on the chromosome numbers of Umbelliferae. III. *Botanisk Tidsskrift*, 42, 384–99. 1933.

A NOTE ON THE FLORAL ANATOMY OF *RIVINA HUMILIS* L.

By EDITH R. SAUNDERS

Sometime Fellow of Newnham College, Cambridge

IN a recent communication to this *Journal* Joshi and Rao have described their investigation of the floral anatomy of *Rivina humilis* L. (1). In the course of a comparison of their observations with my earlier account of certain morphological and histological features of the flower of this species, these authors make various statements which call for some reply.

These authors state (*loc. cit.* p. 360) that my account contains no reference to the mode of origin of the marginal bundles of the perianth members. From this it is evident that they have overlooked my citation of the Rivineae in an article dealing with Angiosperm morphology which appeared in this *Journal* in August 1932 (3). In this article I described the commissural origin of the marginal veins of the tepals in some Rivineae and illustrated the point with figures from *Rivina humilis* (*loc. cit.* Figs. 5-7). Furthermore, I more recently endeavoured to show that petaloid colouring in monochlamydeous types, *when confined to the tepals* is associated with such a mode of origin of the marginal veins, specifically citing *Rivina humilis* among such types (4), p. 208). I have also drawn attention to the fact, which these authors' observations serve to confirm, that the midrib bundles of the four tepals originate at different levels (3), p. 180).

At a later point these authors note that, whereas in my earlier account dealing with the interpretation of the gynœcium of the Rivineae, I have represented the residual vascular tissue after the exit of the stamen bundles, as seen in transverse section, as consisting of four separate groups of differentiated elements, they found in their material at this stage a complete ring. In regard to this difference, if it is a reality (this is rendered uncertain since in their figures xylem, phloem and undifferentiated elements are not distinguished) I would emphasise the point that the number of residual vascular elements at any particular stage will vary with the age of the material. In any case the appearance at this level is not significant and affords no guide to the number of carpels present. For the strands seen at this level are not, as these writers imply that I hold them to be, already defined carpel bundles. Reorganisation, such as occurs ordinarily at any node, has first to take place, as is evident from these authors' figures as well as from my own. It may be added that it is difficult

to conceive how the stamen bundles, composed as they are of both xylem and phloem elements, can leave the central cylinder without causing gaps to appear at some stage in development.

These writers further state (p. 362) that in my account of the gynœcium I have omitted mention of a notch in the outline which is to be seen on the "posterior" side in transverse section, and that, in their opinion, the presence of this notch supports their view that the ovary is composed, not, as I have indicated, of two carpels, but, as has hitherto been supposed, of a single open (=valve) carpel. In regard to this point I would direct attention to the fact that my figures of the gynœcium of the Rivineae (2) are taken from three genera, *Ledenbergia* (Figs. 13-15), *Petiveria* (Figs. 14-19), *Rivina* (Figs. 20-24); that in order to make the series more complete different stages were selected for representation in the different genera; that the ventral indentation in the ovary outline is shown in my figure of *Ledenbergia* (Fig. 14), and for that reason, since the ovary of both genera is constructed on the same plan, an earlier and a later stage were chosen for representation in *Rivina* (Figs. 22, 23).

I entirely concur with these authors, as is clear from my original account, that this indentation corresponds with the line of fusion of the edges of a valve, and I would add, sterile, carpel. *But these edges are only able to meet after the second consolidated carpel which bears the ovule has come to an end.* In all three genera (*Ledenbergia*, *Petiveria*, *Rivina*) the sterile member far exceeds the fertile member in length as can be seen from the difference in level at which their respective main bundles end. A similar appearance, due to the same cause, is to be seen in Nyctaginaceae. This inequality finds a certain analogy in *Epimedium* and *Jeffersonia* (Berberidaceae) where, also, one carpel considerably over-tops the other, the edges of the longer one, as in Rivineae, wrapping round and meeting over the top of the short one, though here it is the fertile member which is the longer (semi-solid type) and the sterile member which is the shorter (valve type). The evidence for the presence of two carpels in the Rivineae, the facts not being in question, appears to me to remain conclusive.

REFERENCES

- (1) JOSHI, A. C. and RAO, V. S. Floral anatomy of *Rivina humilis* L., and the theory of Carpel Polymorphism. *New Phytol.* **32**, 359-63. 1933.
- (2) SAUNDERS, E. R. Illustrations of Carpel Polymorphism. VI. *New Phytol.* **29**, 81-95. 1930.
- (3) — On some recent contributions and criticisms dealing with morphology in angiosperms. *New Phytol.* **31**, 174-219. 1932.
- (4) — The cause of petaloid colouring in "apetalous" flowers. *Journ. Linn. Soc. Bot.* **49**, 199-218. 1933.

REVIEWS

British Economic Grasses, Their Identification by the Leaf Anatomy.

By SYDNEY BURR, M.Sc. and DOROTHY M. TURNER, B.Sc.

Edward Arnold and Co. Demy 4to. 34 plates illustrating 54 varieties. Price 10s. 6d.

The authors have set out to supply a long-felt need to the agrostologist. Attention is being increasingly directed to an exhaustive study of the grasses which compose the turf of our meadows, pastures and sports grounds. As these turves need to be analysed at all times during the year, and since they are often subjected to heavy grazing or cutting, some means of identifying the component grasses other than by their flowers must be found. Perhaps the most widely used handbook up to the present has been Marshall Ward's *Grasses*, which classifies these plants in three different ways, according to vegetative characters, leaf anatomy and "seeds." It has the great advantage of fitting into one's pocket. The present work is more limited in its scope, but goes into greater detail and is fully illustrated by well executed drawings under the microscope. It is intended primarily for laboratory use, and is too large for the pocket.

The book is sent out with a foreword by Professor R. G. Stapledon, C.B.E., M.A., who, with his staff of specialists at Aberystwyth, has done so much to elucidate the problems presented by our grasslands.

In their introduction the authors describe the morphology of the grass shoot and the anatomical structure of the leaf-blade, and give directions as to the method of preparing a suitable hand section. Two keys are then supplied, one utilising the vegetative characters of the shoot, the other the anatomical structure of the lamina. It is intended that these keys should be used together, the one to confirm the other. The main part of the work is arranged so that the grasses follow in alphabetical sequence according to their generic names, with the descriptions occupying one side of the book and the corresponding plates the other. At the end of the book is a glossary incorporating an index to Latin and common names.

As the book comes into general use the keys will be thoroughly tested, and any weak points exposed. Certain points strike one from one's own special knowledge. For instance, in the key to anatomical characters on p. xix the alternatives under C β I i are unfortunate in that quite frequently both *Festuca capillata* and *F. ovina* may have unbroken, sub-epidermal, sclerotic bands. A more uniform difference is to be found rather in the shape of the leaf-sections and in their diameter.

The illustrations form a most useful part of the book, and the authors deserve high commendation for their draughtsmanship, and the publishers for their reproduction. Certain minor points of criticism, however, suggest themselves. In systematic botany we recognise only one type specimen, that of the author responsible for the name of the plant. In practice it is more useful to represent it as an average struck by examination of all the variants. When otherwise wild plants are cultivated wide variations are induced, and one feels that the authors have chosen to draw, it may be designedly since their book is specially intended for those engaged in the cultivation of grasses, from the rather specialised cultivated material, rather than from the average wild plants. Thus the drawings may be somewhat misleading to those concerned with the latter. From Plate XXI the impression is gained that *Festuca rubra* subsp. *fallax* has normally two costae more than subsp. *genuina* and that the shoot is

compressed somewhat differently, as drawn. Actually it is quite impossible to distinguish the two types from anatomical structure alone. Perhaps it would have been as well if the authors had given the source of their material, whether wild or cultivated British, indigenous or introduced strains.

Another point which strikes one in turning over the plates is the variation in magnifications. It would have been much more useful for comparison if the authors had kept to a standard magnification throughout, or, if this were not practicable, at least in the types within a genus or closely related group of genera. Thus, within *Festuca* the magnifications are $\times 150$, $\times 185$ and $\times 225$.

It must have been difficult for the authors to decide what grasses to include and what to omit, but in view of some of their inclusions one wonders why others have been excluded, such as *Glyceria maritima* or *distans*, *Bromus arvensis*, *B. inermis*, *Festuca pratensis* (more important than *F. arundinacea*), *Koeleria cristata* and *Poa serotina*.

Since also the book is designed for scientific use, one feels that it would have been better if the nomenclature had been brought into line with current views, or at least based on, say, the *London Catalogue of British Plants*. Sometimes the accepted name is given as a synonym, but more usually it is omitted. Quite a number of corrections would have to be made; *Agropyron* for *Agropyrum*, *Deschampsia flexuosa* has precedence of *Aira*, *Alopecurus myosuroides* for *A. agrestis*, *Arrhenatherum elatius* for *A. avenaceum*, *Trisetum* for *Avena flavescens*, *Bromus ramosus* for *B. asper*, *B. hordeaceus* for *B. mollis*, *Festuca Myuros* for *F. Myurus*, *Hordeum nodosum* has precedence of *H. pratense*, *Lolium multiflorum* for *H. italicum*, *Ammophila arenaria* for *Psamma arenaria*, *Sieglingia* has precedence of *Triodia*.

We must remember, however, that in publishing this book the authors have broken new ground. There will undoubtedly be a great demand for the work, and the experience gained from its wide use will serve as a guide in the consideration of a further edition. It will make a strong appeal to all those who are working on the various problems related to grassland management. There is no reason why, for example, sports groundsmen, with the minimum of botanical training, should not make it a reference book for their own particular problems, so that they can periodically analyse the gramineous constituents of their greens with a view to the improvement or maintenance of the latter, and as a means of detecting deterioration. There is considerable reason why unprofitable pastures under treatment for improvement should be periodically analysed, for the grasses which are most grazed are less obvious to the casual observer than those which the animals reject, and which therefore grow unhindered and arrive at maturity. If the book stimulates to further intensive and scientific study the activities of all those persons who are engaged with the problems relating to the management of grassland, it will have achieved a success which the authors well deserve, and which one most cordially wishes them.

W. O. HOWARTH.

Elements of Botany. By R. M. HOLMAN and W. W. ROBBINS.
2nd edition. New York: John Wiley and Sons, Inc.; London:
Chapman and Hall, Ltd. 1933. $8\frac{1}{2}$ in. \times $5\frac{1}{2}$ in. Pp. ix+404.
Price 16s. 6d. net.

The textbooks of Professors Holman and Robbins are already too well known to need general description. The present volume is a second edition of their shorter text intended to be digested in a single semester, and is about equivalent to an English one-term course in general botany. Like its predecessors this book is distinguished by the outstanding merit of its illustrations,

the great majority of which do illustrate and illuminate the text, are boldly drawn and reproduced on a generous scale.

In spite of certain changes involving a slight enlargement the subject-matter in this edition remains solidly conventional. The general part is based on a sound groundwork of angiosperm morphology and anatomy with the usual "asides" concerning physiology, while the latter part of the book is devoted to a survey of the major groups of plants. Here the treatment is rather variable; some groups are represented by a "type" (e.g. *Funaria* and *Pinus*) while others are treated more generally (e.g. the green algae, ferns and angiosperms), but the result is a not unfair sketch of the plant kingdom.

Every effort has been made to render the book as easily read as possible. Technical terms have been kept in their proper place and carefully defined where admitted. The dullest student should have the minimum of difficulty in working his way through these chapters.

As the book is prepared primarily for American conditions it may not be out of place to note a few points where it might be difficult to adapt to English needs. The student would need to remember that the United States had their civil war in 1860; to read U.S.A. for "this country" and maize for "corn." He would also be puzzled to identify Joshua trees, black locust, porcupine grass, cottonwood, Canada thistle, Johnson grass, morning glory and others. Among the types described more fully *Riccia* is not often used in English elementary courses, and *Pellia* is generally preferred to *Marchantia*. These points are perhaps trifling, and apart from the fact that the price is decidedly higher than is usual for elementary textbooks here, there is little more to be mentioned. There is an almost complete absence of unfamiliar idiom.

The binding is of cloth impregnated with pyroxylin which the publishers claim renders it immune to spoiling by water or vermin. The protection against water should make it particularly suitable for laboratory use.

W. O. JAMES.

An Index to the Genera and Species of the Diatomaceae and their Synonyms, 1816-1932. By FREDERICK WM. MILLS, F.L.S., F.R.M.S. London: Wheldon and Wesley, Ltd. 1933. Price 10s. per part.

This important work, which is being issued to subscribers, is appearing in monthly parts. It will be completed in about twenty parts, of which six have already been issued, and will form three volumes.

The parts already issued point to this being one of the most important modern contributions to the literature on the Diatomaceae.

It was originally undertaken by the author for his private use, but it is fortunate that he has been persuaded to publish the vast amount of information which he has collected.

The work is not only an index of genera and species but is an attempt to relegate synonyms to their proper places. The alphabetical arrangement should facilitate ready reference. The treatment of synonyms is, particularly in the Diatomaceae, a matter of considerable difficulty, but the author has adopted a *via media* which does not, on the one hand, carry condensation too far, nor, on the other hand, recognise the validity of questionable synonyms and insufficiently defined species and varieties.

Where such difficult problems as the values of species and varieties, the priority of names, and the collation of synonyms have to be dealt with, it would be too much to hope that anyone could settle all questions beyond dispute, but Mr Mills has certainly cleared up quite a number of points and has, by his generous references, made verification a comparatively easy matter.

A reference to any genus makes it evident that the author has carefully compared an enormous mass of diatom literature and has judiciously condensed the collated information. In future it should not be difficult to examine and compare forms previously figured and described with any form supposed to be new. The bibliography is extensive and should prove of great value. Those interested in the systematic study of the Diatomaceae are badly in need of a standard catalogue and Mr Mills' work goes far to provide for that want. The index is not without errors, but these should not seriously detract from a work which no serious student of diatoms can afford to neglect.

DAVID MCCALL.

The Methods of Cellulose Chemistry. By CHARLES DOREE, M.A., D.Sc., F.I.C. London: Chapman and Hall, Ltd. 1933. Pp. x + 499, with 67 figures. 21s.

This is rather an unusual kind of book. The author had the very laudable ambition of providing not a textbook on the chemistry of cellulose and its derivatives, but a textbook of methods employed in the experimental investigation of cellulose in many of its manifold uses and modifications. There is in Parts I and II much of interest to the industrial chemist. The whole subject of applied cellulose chemistry is an extraordinarily broad one, the commercial processes involving in some form or another this, the fundamental constituent, of the plant world are too many to estimate. It is, however, very true that "one man's cellulose is another man's poison," as a result of which the subject is a very divergent one. Methods which may be suitable and valuable in one field or for one derivative are useless and valueless in another. Because of this, and because many of the methods ordinarily employed have been decently interred in trade journals and are therefore somewhat difficult to exhume, this book is an important one. Dr Doree is to be congratulated on his patient efforts in assembling these very full abstracts of original investigations. Full working details are given, and while this does not entirely replace the original paper, the reading of them may often suggest ideas for further research.

Part III is rather more ambitious. In it the author attempts to describe methods for the investigation of cellulose as it is found *in situ* in plant tissues and wood, and of the various other constituents such as hemicelluloses and lignin which may be found along with it. In this he is not quite so successful, perhaps because the original intention of providing a summary of methods has been sometimes forgotten. While it is always interesting, it is at times indifferently "textbooky," as for example in the chapter on the chemistry of isolated lignin, and at times rather aimless, as for example the chapter on hemicelluloses. In view of the strongly applied bent which the first part of the book has, it is rather surprising that more attention was not given to the analysis of woods and to the methods of evaluation of pulps. The final chapter on the pectic substances is interesting but a little out of place in a book on cellulose methods. There is no reliable evidence for the existence of a pectocellulose as so frequently postulated, and it does not clarify the issue to describe a "pectin-cellulose complex with a constitution analogous to that of the glucosides."

To sum up, it can be stated again that this is an unusual book, and an unusually valuable book to a wide circle of workers on all phases of cellulose chemistry. It may perhaps help to remove some of those barriers which seem to erect themselves so easily between the different sections of a diverse subject of this sort.

A. G. NORMAN.

Recent Advances in the Study of Plant Viruses. By KENNETH M. SMITH, D.Sc. Pp. xii + 423, with coloured frontispiece and 67 text-figures. London: J. and A. Churchill. 1933. Price 15s.

It is 41 years since Iwanowski showed that the juice of diseased tobacco plants was still infective after it had passed filters which retained-bacteria. At the time the observation attracted little attention, but Beijerinck's provocative theory of a *contagium fluidum vivum* and the demonstration that filterable viruses occurred also in animal diseases started a literature which has gone on increasing in volume, until its flood threatens to submerge all but a few of the specialists on the subject. On the animal side the importance of the new discovery to human pathology ensured rapid progress from the first, and research has been continuous and fruitful. On the plant side there was a long period, nearly 20 years, of comparative inactivity until the work of Allard in America gave a new stimulus and started the eager research whose quantity is now so overwhelming. Memoirs on particular aspects have been published from time to time, and a few comparatively brief summaries of the whole subject, but there has been no comprehensive work covering most of the field of plant virus research until the present volume by K. M. Smith. It fills a gap, therefore; and it fills it very well.

The volume, which is of convenient size and well supplied with illustrations, is intended as a survey of the knowledge at present available on plant viruses, and also as a reference book which may serve both the general student and the research worker until the progress and correlation of knowledge allow a more comprehensive treatise to be written. This dual or triple purpose has led, no doubt inevitably, to a certain lack of balance, the great detail in certain sections contrasting oddly with the want of it in others. Perhaps, too, British contributions to the subject, important as they unquestionably are, receive a greater relative prominence than is strictly their due. But these are minor points readily adjustable in the later editions which will certainly be called for, and they detract hardly at all from the great excellence of the book as a whole. It is an admirable, trustworthy, readable survey, and its value to the plant pathologist is enhanced by the prominence given to the contributions made by the workers on human and animal pathology, whose researches are apt to escape his notice. Eleven chapters are given to a general account of the symptoms, properties, transmission, physiology and, as we might hope and expect from the writer, to a full treatment of the relations between insects and the diseases they carry. These are followed by three chapters describing the various diseases classified according to the hosts they attack; and to every chapter is appended a bibliography which is unusually excellent.

J. HENDERSON SMITH.

THE NEW PHYTOLOGIST

VOL. XXXIII, No. 2

28 APRIL, 1934

DUKINFIELD HENRY SCOTT

1854-1934

By the death of D. H. Scott on the 28th January of this year we have lost a great botanist who was endeared to generations who knew him by his sterling and simple nature. At the time of his death, though perhaps a little frail physically, he was otherwise enjoying his full powers, and had life been prolonged would have continued to contribute researches in fossil botany of the high standard he had created. The sense of loss is mitigated by two circumstances. He leaves behind him an almost faultless achievement in his own field; whilst for his fellow botanists and other friends there is the indelible impression of his unique personality.

The bare facts of Scott's life are simple. Thrown on the world from Oxford with a non-science degree and a competency, his youthful studies had inspired in him the wish to follow botany. Carpenter's *The Microscope and its Revelations* and Henfrey and Griffith's *Micrographic Dictionary* ("that was the book that brought me into botany"), with translations of Hofmeister, v. Mohl and Naegeli—these he had read, possibly under maternal guidance. He found his way to Thiselton-Dyer (about 1879) for advice as to the next step. With the concurrence of Vines this led to Sachs and Würzburg, a phase delightfully recorded by Scott in reminiscences printed in this *Journal* (1925). On his return in 1882 my father welcomed the chance of placing him in charge of the practical classes in botany at University College, London, with a free hand, in succession to F. O. Bower. Here I came under him as a student and was taught proper respect for the subject in the following way.

A fellow-student and I discovered that on certain mornings the botanical laboratory was unoccupied and we used to repair to this quiet spot to solve together mathematical and physical exercises. On one occasion Dr Scott came in earlier than usual and as he passed turned to enquire what we were doing, and discovering the nature of our task passed on to his little cubicle without a word. We realised at once the enormity of our crime, and that this slip of a laboratory, filched from a corridor, was in reality a Temple of Botany, a Holy Place for particular rites. We never transgressed in this respect again. I should think throughout life it was rarely necessary for Scott to reproach anybody, or expostulate, in a matter of conduct. He was held in such universal reverence and affection that nobody about him would dream of doing anything of which he might disapprove.

In 1885 he transferred to the Royal College of Science as Assistant Professor under Huxley. It was at this time he had the happy inspiration to secure the sanction of Thiselton-Dyer (now Director at Kew) to bring his advanced class to work in the Jodrell Laboratory on certain days. Sanction may perhaps have been grudgingly given, but it was given, and for Scott's career it was to prove momentous, and for two reasons. The first batch of students from South Kensington included Miss Rina Klaassen whom Scott married in 1887. This marriage was the foundation of the happy home life so essential to his nature. It was indeed an ideal union. Secondly, this arrangement at the Jodrell paved the way to Dyer's inviting Scott in 1892 to become Honorary Keeper of the Laboratory, a position he retained for fourteen years. Here, to the lasting advantage of botany, Scott became the inspiring centre of research for the younger generation, especially in the fields of anatomy, morphology and palaeobotany. This was the period during which Scott consolidated his position and attained the recognition and ascendancy proper to a man of his quality. The secrets of Scott's influence were simplicity and directness, strong feelings and the gift of expression, combined with unerring and sober judgment. His mind was clear and adaptable—never finally closed on any scientific matter. Extravagant hypotheses brought a douche of cold water. Even before he reached eminence in his chosen field of fossil botany he was admitted to the Royal Society (1894), which later conferred on him the Royal and Darwin medals. He also became an Editor of the *Annals of Botany*, a connection which lasted till his death.

We may now turn to the field of fossil botany which as a scientific study owes a very great deal to Scott. It happened that in 1889 Prof. W. C. Williamson, who had been industriously describing coal measure petrifications for twenty years and had reached the age of seventy-three, was looking for a younger botanist versed in anatomy who might succeed to his mantle. It was at the meeting of the British Association at Newcastle-on-Tyne in 1889 that Williamson heard Scott read a paper "On some recent progress in our knowledge of the Anatomy of Plants." This convinced him that Scott was his man. One sentence may be quoted: "It need scarcely be pointed out how important an adequate knowledge of anatomical characters should be from a palaeontological point of view." Scott was ignorant of what was impending. The three papers ("Further Observations") which emerged from the collaborations of Williamson and Scott in the earlier 'nineties marked Scott's apprenticeship in palaeobotany. They were definitely written by Scott himself ("every word") and represent his own conclusions at the time. Williamson died in 1895 and Scott always referred with pleasure and gratitude to this contact as being one of the most important of his life.

In 1896/7 Scott gave his famous course of lectures on Fossil Botany at University College, London. Widely attended, they appeared in 1900 as his *Studies*, a book which transformed the subject and has become a "classic."

All this time he was deeply engaged in research—new types such

as *Cheirostrobis*, *Medullosa anglica* and *Lepidocarpon* came under his hands and formed the subjects of important monographs. He was well served by the collectors, Lapidaries, Lomax and Hemingway, by the cameras of Boodle and Tams, and by the faithful drawings of Gwilliam. His wife, a trained botanist, assisted him in many technical ways. Everybody held it a privilege to be associated with Scott in whatever capacity.

It once fell to my lot to collaborate with Scott, and under the following circumstances. Amid the enthusiasm for fossils aroused by his 1896/7 course I procured numerous slides for the University College Collection. Finding that French silicified material was not available in England I visited Autun Grand'Croix, and other French localities, and returned with numerous uncut blocks. As these were being sectioned by Lomax and Krantz, I found many detached seeds beautifully preserved. I showed a selection to Scott (about 1901) and he too was impressed. I said I was getting interested in these things and meant to go on with them, and if possible discover their origin. Scott gave his blessing though on reflection it seemed to lack cordiality. As the investigation progressed I had the curiosity to consult the rich Williamson Collection, now in the Natural History Museum, thinking a few French specimens might have strayed in by exchange. But the English Coal-Measure stuff proved too great a temptation, and I stumbled on a number of small things which added together satisfied me that the seed *Lagenostoma Lomaxi* was borne by the well-known fossil *Lyginopteris* (then *Lyginodendron*) *oldhamium*. But I had an uncomfortable feeling, as the English fossils were tacitly Scott's hinterland. For the Williamson Collection had gone to the Museum through the generosity of Scott who had put up the purchase money, the Museum repaying him by annual instalments according to their resources. Had he not done so the collection would have gone abroad and the sequel would have been different. I felt like a poacher in the coverts of a friend, and I also bethought me that possibly Scott had intentions on the seed problem himself, and was inhibited from following them by the numerous undescribed types which were reaching him from the pits. So I drafted an account of my observations and conclusions, and took it to Scott at the Jodrell. He also looked at the critical preparations.

Raw as I was as a palaeobotanist, I had been well enough trained by Scott to realise the magnitude of the discovery, and that it would bring a large number of palaeozoic "ferns" into the category of seed plants—the group we eventually named Pteridosperms. It was evident to me as a relative novice that I should have difficulty in making the discovery acceptable to the botanical world, and this weighed as an additional reason for bringing Scott in. So I suggested we should pool our resources and make a joint work of it—an unconventional approach, the lesser luminary inviting the great man to collaborate. On this occasion he declined with the words "there is nothing left but the shouting!" The next time I came I had elaborated the case for joint authorship and he consented. This piece of collaboration extending over two years was a very great pleasure

to me. All who have worked with Scott will know what it is to be treated as an equal by such a man. And so the *Phil. Trans.* paper of 1904 took shape. With the lead thus given many other cases poured in from Scotland, France and America. Neither Scott nor I ever wavered, but it was twenty-six years later that W. J. Jongmans brought to the International Botanical Congress at Cambridge slabs of impressions of *Sphenopteris Hoeninghausi* from Holland covered with attached cupules that would have convinced even the man in the street.

We botanists may think of Scott as one of ourselves, but he never ceased to belong to the Scotts, a numerous clan, bristling with talent. He was as faithful to them as to us, and loved to circulate to their marriages and gatherings just as he did on scientific occasions. He was a large-hearted man intensely interested in human affairs. Put him alongside a woman of the world with a spice of frivolity and you would discover another Scott, equally scintillating, in this different field. He loved a gossip.

As President (Linnean and other Societies, Congresses, B.A. Sections) he was in great request, and felicitous both on ceremonial and less formal occasions. His lectures were delightful and he had the unconscious habit of trying to climb up his pointer—once I saw it break, reminding me of his friend Count Solms of whom they said, "It was no lecture unless he broke a lampshade or two."

Two passages may be quoted from the last letter I received from him about a month before he died. "I am very lazy: my job just now is to write a chapter on Fossil Pteridophytes for a Dutch book (Verdoorn's) on Pteridology. I'm rather fed up with boil-downs, and would rather be doing a little fresh work to finish up with." And, excusing himself for not attending a meeting, "I didn't go, as the weather was too cold and foggy for me. I have now entered on my 80th year, and am disposed to take a little care of myself. The boys are sliding on the ponds, a few leaves stick on the oaks still; how do deciduous trees behave in Egypt?"

Had he lived in the country where these lines are being written (Egypt) he would have been a great sheikh, and a commemorative tomb would have been built in some remote desert spot where the faithful would foregather on suitable occasions.

F. W. OLIVER.

ANATOMY OF *SILENE VULGARIS* AND *SILENE MARITIMA* AS RELATED TO ECOLOGICAL AND GENETICAL PROBLEMS

I. ROOT STRUCTURE

By M. ETELWYN MILLNER, M.Sc., F.L.S.

(With 19 figures in the text)

I. INTRODUCTION

THE investigation of the anatomy of *Silene vulgaris* and *S. maritima* has been started with the object of making a comprehensive study of the vegetative and reproductive structures of two species of *Silene*, their variations and hybrids, in connection with genetical and ecological researches being conducted at the Royal Botanic Gardens, Kew, and at the Potterne Biological Research Station. The work will involve correlating the anatomical structures found with genetical results and with a wide range of environmental conditions.

The present paper deals with the specific structural differences in the roots of *S. vulgaris* and *S. maritima* and with anatomical fluctuations due to edaphic factors in the roots of *S. vulgaris*. Transplants of the latter, grown on the experimental soils at Potterne, show slight modifications in root structure correlated with the type of soil. These soils are sand, calcareous sand, clay, calcareous clay, and Potterne reserve soil as described in the "Report on the Transplant Experiments of the British Ecological Society" (3).

Little work appears to have been done on the anatomy of roots of *Silene*. Hill(1) in describing the seedling development of certain species of *Silene* mentions the diarch structure of their young roots. Solereder(5) refers to the large amount of unlignified tissue in the secondary xylem, as characteristic of the Caryophyllaceae.

II. EXTERNAL MORPHOLOGY AND HABIT

(a) *Silene maritima*. Arising from the crown is a varying number of main roots which branch, the number of main roots and branches depending on the age of the plant. A fairly young plant is shown in Fig. 1 A. Here the main root has stopped growing and a number of branches arise about an inch below the crown. The branches are quite

smooth, the main root being slightly wrinkled. The roots ran straight down in the soil of the Kew experimental beds. The majority of the roots of all *S. maritima* plants examined are quite smooth, but the older, about half an inch in diameter, are wrinkled slightly (Fig. 1 A).

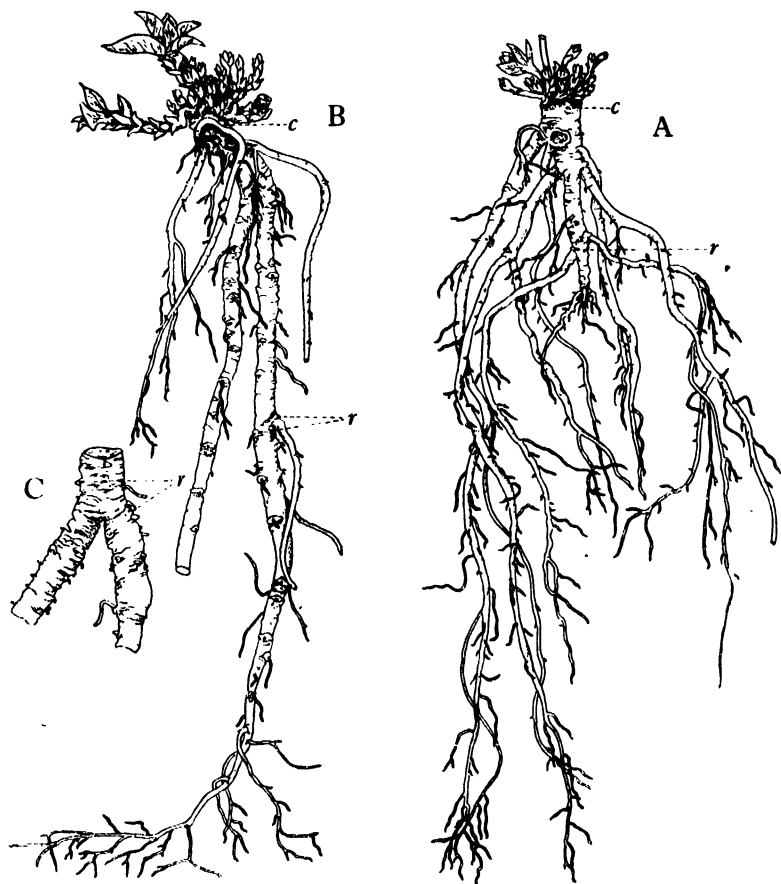


Fig. 1. Roots of *S. vulgaris* and *S. maritima* showing mode of growth. A, *S. maritima* ($\times \frac{3}{8}$); B, *S. vulgaris* ($\times \frac{3}{8}$); C, small portion of root of *S. vulgaris* to show wrinkling ($\times \frac{3}{8}$). c, crown; r, ridges produced by emergence of lateral roots.

(b) *Silene vulgaris* shows the same general type of root system as *S. maritima*, but the wrinkling is more pronounced. In fairly young roots the ridges on the surface seem in every case to be present around a lateral root, or marking the position of a withered one (Fig. 1 B), but in older roots there appear to be wrinkles other than those caused by the emergence of laterals (Fig. 1 C).

The actual details of the root system vary however with the type of soil, the plants in any one soil showing uniformity in their root systems. The material examined was collected from roots as follows [to quote from the "Second Report on the Transplant Experiments of the British Ecological Society" (4)].

Sand. About five main roots branched 8–10 cm. below the crown, and then ran straight down without forking to a depth of 5–7 dm. The superficial colour of the washed roots was "Clay Colour¹."

Calcareous sand. About seven main roots, with little or no branching, ran straight down to a depth of 6–7 cm. The superficial colour of the washed roots was "Antimony Yellow¹."

Clay. About twelve main roots, three or four of which ran straight down, but had decayed at 20–25 cm. depth. The remainder spread horizontally 4–5 cm. below the surface of the soil and to a distance of 6–7 cm. or more from the crown. A good deal of branching occurred at various distances from the crown. The colour of the roots was "Chamois¹."

Calcareous clay. About twenty-four main roots, with little or no branching, ran straight down to a depth of 7 dm. The superficial colour of the washed roots was "Cream Buff¹."

Potterne soil. About four main roots were very much branched 5–8 cm. below the crown into approximately thirty branches. These were at first coiled into a bunch and then ran approximately straight down. The superficial colour of the washed roots was "Antique Brown¹."

III. ANATOMY OF TYPICAL ROOT OF *SILENE VULGARIS*

(a) *Primary structure.* As secondary growth takes place at a very early stage seedling roots, half an inch long, were examined. These have a piliferous layer bearing numerous simple unbranched root hairs averaging 0.25 mm. in length. There is a cortex, four cells in depth, the cells of which are more or less rounded in shape. Within the endodermis and pericycle is a diarch stele of about four metaxylem vessels with protoxylem vessels and a distinct phloem group on either side (Fig. 2).

(b) *Secondary structure.* The primary structure appears soon to be replaced by secondary tissues, so that in roots as small as 2 mm. in diameter the diarch xylem is entirely obliterated. An occasional primary xylem element can be detected in longitudinal sections by

¹ These names are used by Ridgway, *Colour Standards and Nomenclature* (Washington, 1912), with whose plates the living roots were matched.

the annular or loose spiral thickening in contrast to the reticulate thickening of the secondary xylem.

A phellogen arises at a very early stage which forms a periderm, varying in thickness and degree of suberisation according to the soil in which the root is growing. Several layers of phelloderm are also present in older roots. These tissues as seen longitudinally appear to have an irregular line bulging here and there in correspondence with

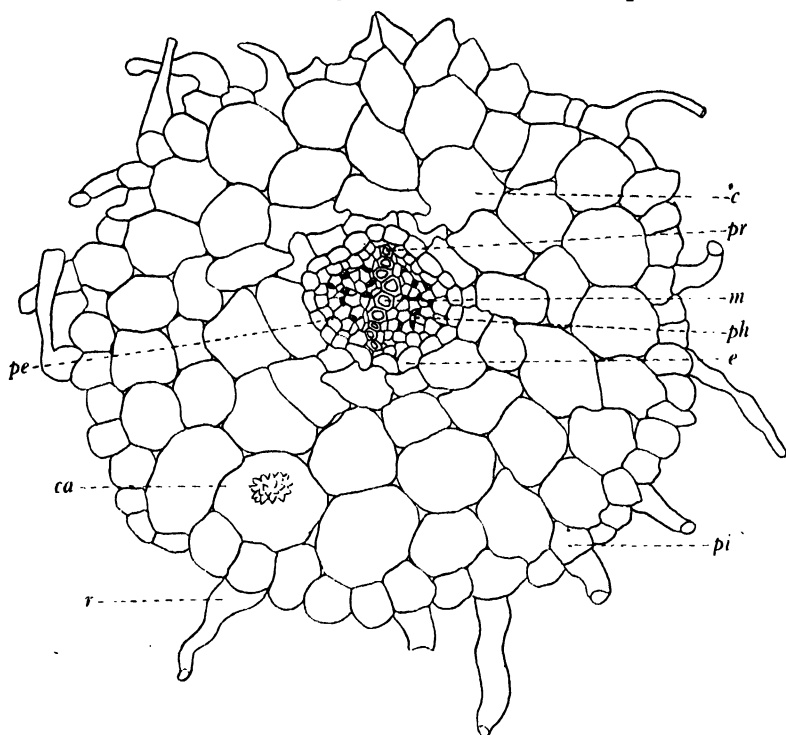


Fig. 2. Transverse section of primary root of *S. vulgaris* ($\times 200$). *ca*, calcium oxalate crystals; *c*, cortex; *e*, endodermis; *m*, metaxylem; *pe*, pericycle; *ph*, phloem; *pi*, piliferous layer; *pr*, protoxylem; *r*, root hair.

the external wrinkles described above, this being especially marked over the place of origin of a lateral root (Fig. 11). Cluster crystals of calcium oxalate are present in the cells of the cortex and also in the parenchyma cells of the centre of the root and in the medullary rays. The crystals appear to be most numerous in the parenchyma cells in the neighbourhood of a lateral root, where they form a thick deposit all around a lateral root from the centre of the root to the cortex (Fig. 11).

The secondary phloem shows no structural peculiarities, being a band of four to five cells in depth arranged in regular radial rows, separated by wide medullary rays (Fig. 5). There is a large quantity of parenchyma among the phloem cells. The xylem is characterised

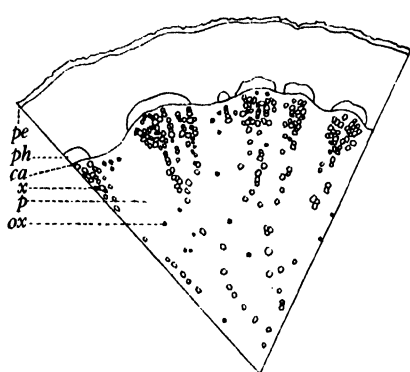


Fig. 3.

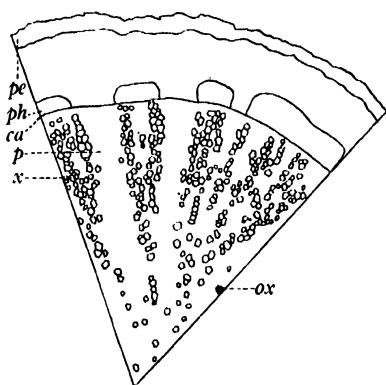


Fig. 4.

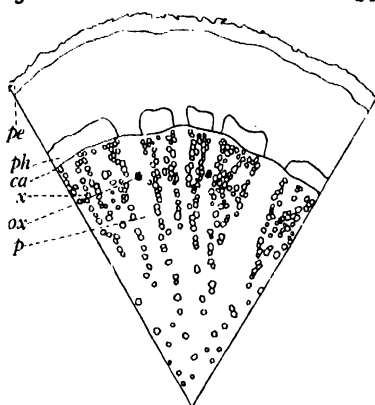


Fig. 5.

Fig. 3. Transverse section of *S. vulgaris* root grown on clay ($\times 7.3$).

Fig. 4. Transverse section of *S. vulgaris* root grown on sand ($\times 7.3$).

Fig. 5. Transverse section of *S. vulgaris* root grown on Potterne soil ($\times 7.3$).

ca, cambium; ox, calcium oxalate crystals; p, parenchyma ray; pe, periderm; ph, phloem; x, xylem vessels.

by the large quantity of unligified tissue which appears to be of the nature of storage parenchyma. The cells of the latter are much enlarged in the centre and are grouped in a radiating manner of five to eight cells around a single vessel (Fig. 6). These cells appear to form a ground tissue in which the vessels are isolated. Towards the cambium the vessels are arranged on radial lines, separated by radial

rows of small unligified cells (Fig. 7). This tissue is useful for the storage of food reserves in the form of glucose and protein, the latter being present in minute grains. These reserves are more pronounced in the centre, where the cells are larger, the protein grains being more numerous in the large cells and the glucose reaction more marked.

In longitudinal aspect the vessels show a peculiar feature, those in the centre have an extremely wavy course bending around the large parenchyma cells (Figs. 9 and 10). The primary vessels with spiral and annular thickening are extremely bent and twisted, as are also the most central secondary reticulate vessels. The bends in the

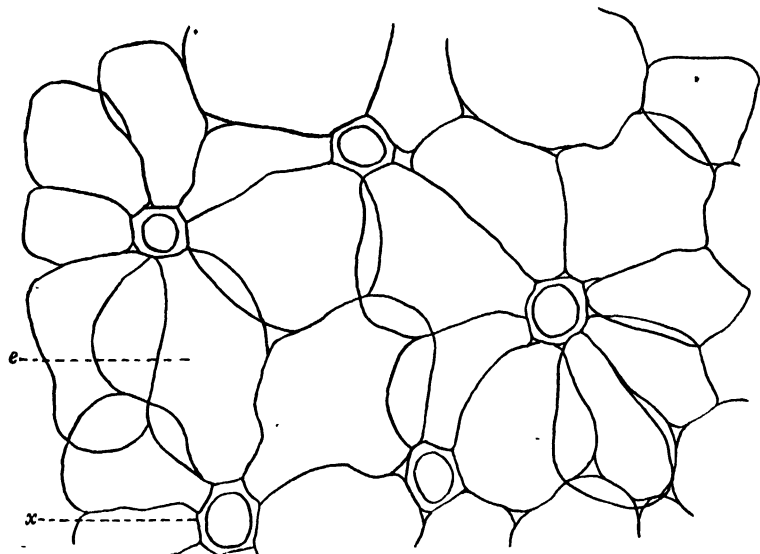


Fig. 6. Transverse section of central xylem of an old *S. vulgaris* root, showing large parenchyma cells ($\times 200$). *e*, enlarged food storing cell; *x*, xylem vessel.

latter appear to be most pronounced at the points where the remains of the transverse septa are still visible (Fig. 10). Those vessels, however, which have only recently developed from the cambium, are practically straight, the vessels becoming progressively straighter from the centre towards the periphery. These straighter vessels correspond with the outer ones arranged in the regular rows and separated by only small parenchyma cells as mentioned above, while the vessels with a wavy course are always associated with the large parenchyma cells and are widely separated from one another.

This twisted course, though less marked, is found in roots in which secondary growth has only just commenced and is more and

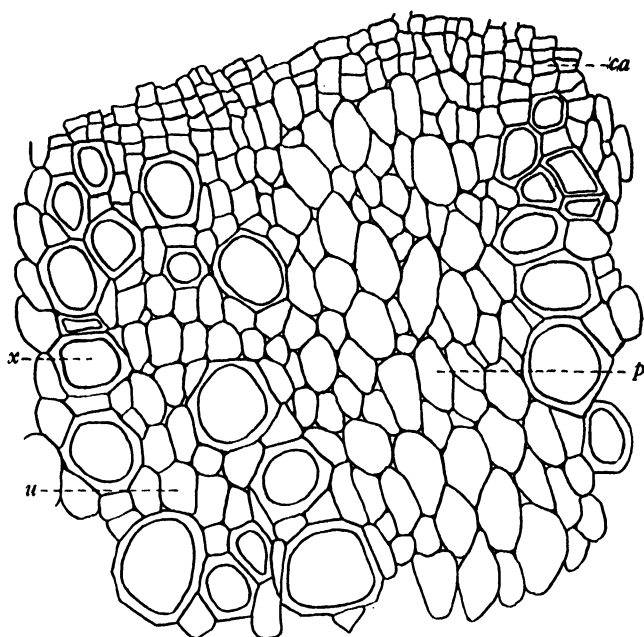


Fig. 7. Transverse section of outer xylem elements of same *S. vulgaris* root as Fig. 3, showing small parenchyma cells ($\times 200$). *ca*, cambium; *p*, parenchyma ray; *u*, unlignified parenchyma; *x*, xylem vessels arranged in regular rows.

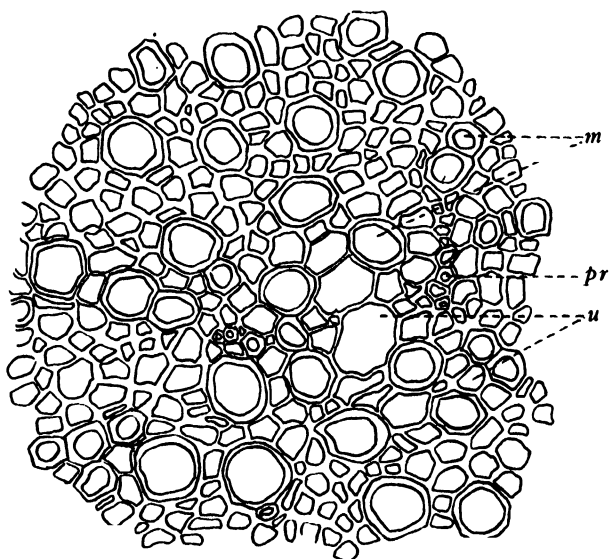


Fig. 8. Transverse section of central xylem of a young *S. vulgaris* root, showing small parenchyma cells ($\times 266$). *m*, metaxylem vessels; *pr*, protoxylem vessels; *u*, unlignified parenchyma.

more pronounced in progressively older roots. Fig. 8 shows the condition of xylem in a young root after secondary thickening has commenced, but before any of the parenchyma cells have enlarged. Here these cells are small, thick-walled, with little food reserve. As the root gets older, these cells enlarge to the size shown in Fig. 6, where

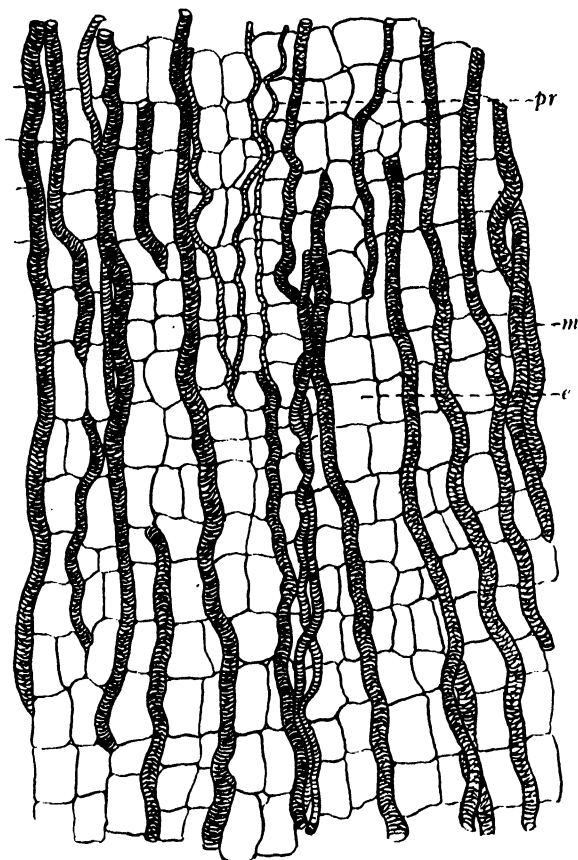


Fig. 9. Diagrammatic longitudinal section through the xylem to show course of the vessels ($\times 46.6$). *e*, enlarged food storing cell; *m*, metaxylem vessel; *pr*, protoxylem vessels.

they are distended with sap and have very thin walls. It would therefore appear that, as the roots get older, the vessels are caused to bend, as the parenchyma cells are enlarging probably with stored food and the vessels are thus separated. As each addition of new xylem is formed there is a tendency for the vessels to become gradually distorted with the progressive enlargement of the storage

parenchyma. It does not seem that this process could go on indefinitely, as roots are rarely found more than half an inch in diameter, secondary growth only taking place up to a certain size.

A further distortion in the course of the vessels is seen at the origin of a lateral root. The vessels appear to bend towards the periphery from above and below the lateral, as if some force exerted from near the centre had pushed them towards the outside (Fig. 11).

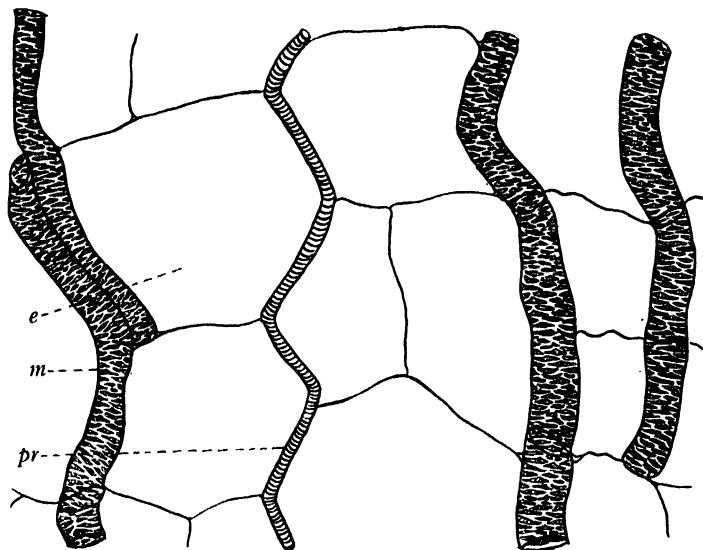


Fig. 10. Longitudinal section of central xylem of *S. vulgaris* root, showing the nature of the vessels ($\times 200$). *e*, enlarged food storing cell; *m*, metaxylem vessel; *pr*, protoxylem vessel.

IV. STRUCTURAL DIFFERENCES IN THE ROOTS OF *SILENE MARITIMA* AND *SILENE VULGARIS*

The primary structure of the roots of the two species is identical and it is impossible to distinguish them from one another by their young roots.

As secondary growth proceeds the differences in the two roots become apparent as slight modifications occur in the xylem of *S. maritima*. The periderm and cortical tissues are alike, save that in the latter they are small, more compact, and more regularly arranged. Calcium oxalate crystals are present. The phloem differs in *S. maritima*, in that it forms a continuous band, medullary rays being absent.

The most striking difference is in the greater number of vessels

in proportion to unligified parenchyma in roots of the same diameter (Figs. 12 and 14). In all sections examined *S. maritima* has far more vessels than *S. vulgaris*. Those vessels nearest the cambium are not

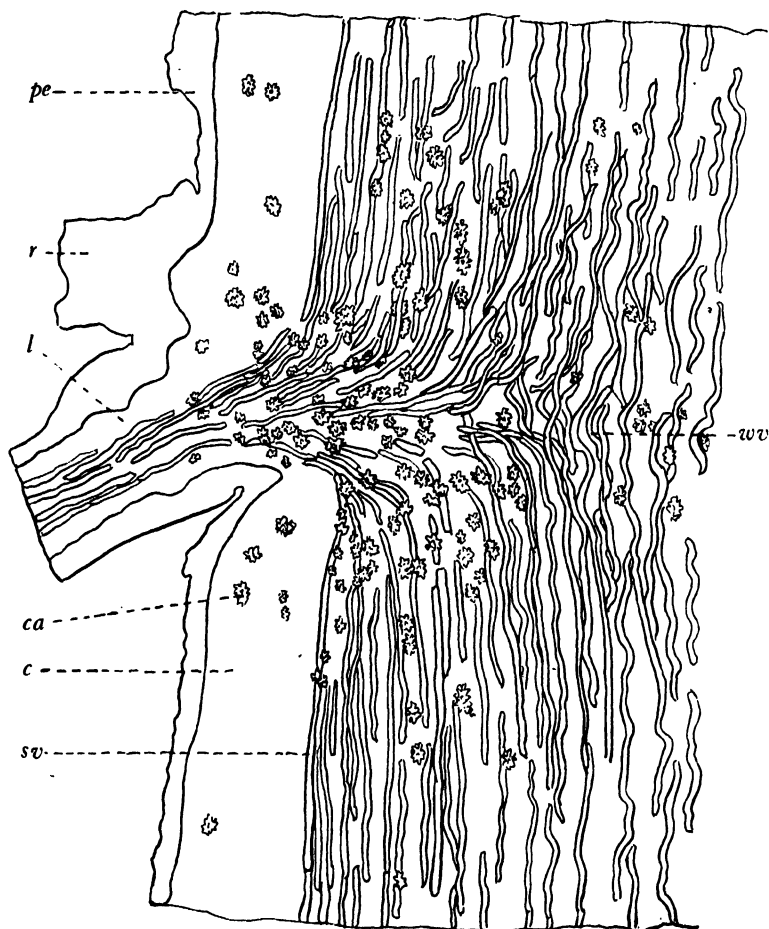


Fig. 11. Longitudinal section of root of *S. vulgaris*, showing the origin of a lateral root and the peculiar course of the vessels ($\times 26.6$). *c*, cortex; *ca*, calcium oxalate crystals; *l*, lateral root; *pe*, periderm; *r*, ridge caused by growth of lateral root; *sv*, straight outer vessels; *wv*, wavy inner vessels.

arranged in radial lines as in *S. vulgaris* but are separated from one another by one or two rows of unligified parenchyma cells. The latter are much smaller and more compact (Fig. 14), the rows varying in extent from two to many cells. The xylem, formed of vessels and unligified parenchyma, forms a continuous tissue, medullary rays

being entirely absent. It is this difference which causes the marked contrast in the appearance of the transverse sections of the roots of the two species.

The tissues in the centre of the roots are more alike, *S. maritima* having the same radiating arrangement of large parenchyma cells

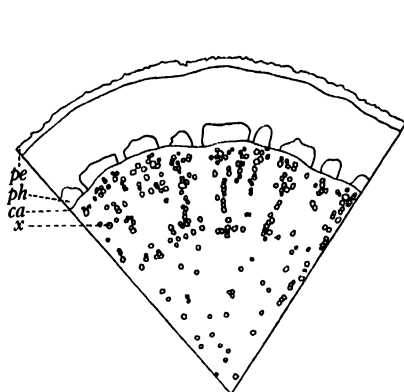


Fig. 12.

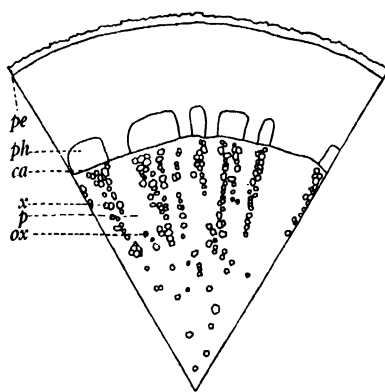


Fig. 13.

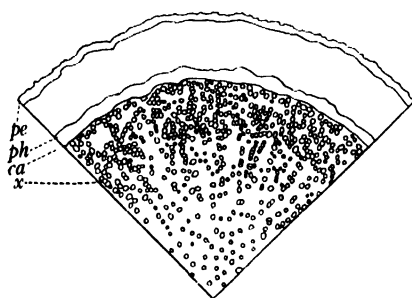


Fig. 14.

Fig. 12. Transverse section of *S. vulgaris* root grown on calcareous clay ($\times 7.3$).

Fig. 13. Transverse section of *S. vulgaris* root grown on calcareous sand ($\times 7.3$).

Fig. 14. Transverse section of *S. maritima* root ($\times 7.3$).

ca, cambium; ox, calcium oxalate crystals; p, parenchyma ray; pe, periderm; ph, phloem; x, xylem vessels.

around isolated vessels (Fig. 14). The two roots are alike in their food reserves, small protein grains and glucose being also found in the parenchyma of *S. maritima*, though the sugar appears to be present in larger quantities than in *S. vulgaris*.

In longitudinal sections the two roots show the same features. The vessels in *S. maritima* have the same wavy course, which is just as pronounced, even though the roots of *S. maritima* are smoother

than *S. vulgaris*. The younger vessels nearest the cambium are straighter, the older vessels becoming bent as they become separated from one another by the enlarging parenchyma cells as in *S. vulgaris*.

There is, however, a further difference in the differentiation of annual xylem. In *S. maritima* there is a definite distinction between each year's tissues. The vessels are few in the centre becoming more numerous towards the cambium, where the difference between spring and autumn wood is clearly marked. The material from which the section in Fig. 14 was taken was collected in June, and the spring vessels are nearest the cambium. The autumn wood consists almost entirely of unlignified parenchyma, which separates the vessels of two seasons. Owing to the peculiar enlargement of the parenchyma cells in the centre, the seasonal differentiation is not apparent in this region. In *S. vulgaris*, however, it is almost impossible to distinguish the tissue of one year from that of another, save perhaps that the vessels are again more numerous in the younger xylem towards the cambium, becoming gradually fewer towards the centre in the oldest xylem (Fig. 3).

V. ANATOMICAL FLUCTUATIONS DUE TO EDAPHIC FACTORS IN THE ROOTS OF *SILENE VULGARIS*

There are slight structural variations in the roots of *S. vulgaris* which appear to be related to the soil in which the root is growing.

In describing the external morphology of these roots colour differences were mentioned. The internal structure of the roots shows that these differences in colour are due to the amount of periderm present and to the degree of suberisation. Those washed roots from clay soil, described as "Chamois" colour, have only three to four layers of periderm, of which only the outer is suberised (Fig. 15). Roots from sandy soils, however, "Clay Colour," have as many as eleven to twelve layers of cells (Fig. 16). These are small, tightly packed, and the outer six to eight layers are strongly suberised. This would account for the darker colour. Between these two extremes, the "Antique Brown" roots grown in the Potterne soil have from six to eight layers of periderm cells, the outer four or five of which are suberised (Fig. 17). The "Cream Buff" roots from calcareous clay have four to five layers, of which only one or two are suberised (Fig. 18), while the calcareous sand roots, "Antimony Yellow" in colour, have from four to six periderm layers, the outermost and part of the next layer being suberised (Fig. 19). Of the last two, which more closely resemble each other in colour and degree of phellogen

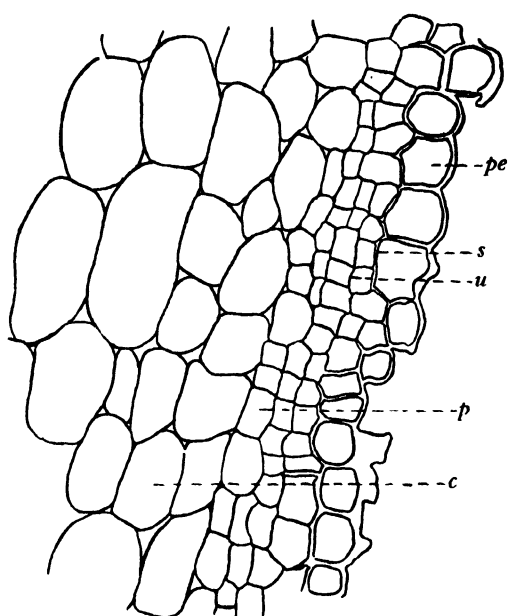


Fig. 15. Transverse section through periderm of root grown on clay ($\times 266$).
c, cortex; *p*, phellogen; *pe*, periderm; *s*, suberised wall; *u*, unsuberised wall.

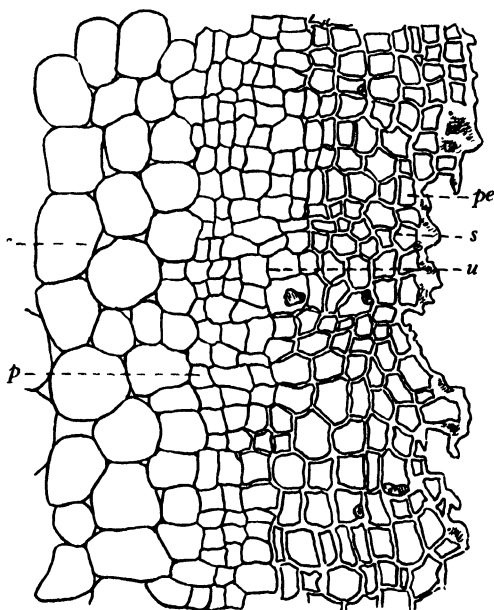


Fig. 16. Transverse section through periderm of root grown on sand ($\times 266$).
c, cortex; *p*, phellogen; *pe*, periderm; *s*, suberised wall; *u*, unsuberised wall.

activity, the cells are slightly smaller and more compact in the calcareous clay roots, though the outermost layer is more irregular and strongly suberised in the calcareous sand roots.

There may be a possible relation between the water content of the soil and the degree of periderm formation, since there is greater formation of corky tissues in roots from the drier sandy and Potterne soils than in the clay. Similarly the extent of suberisation may be

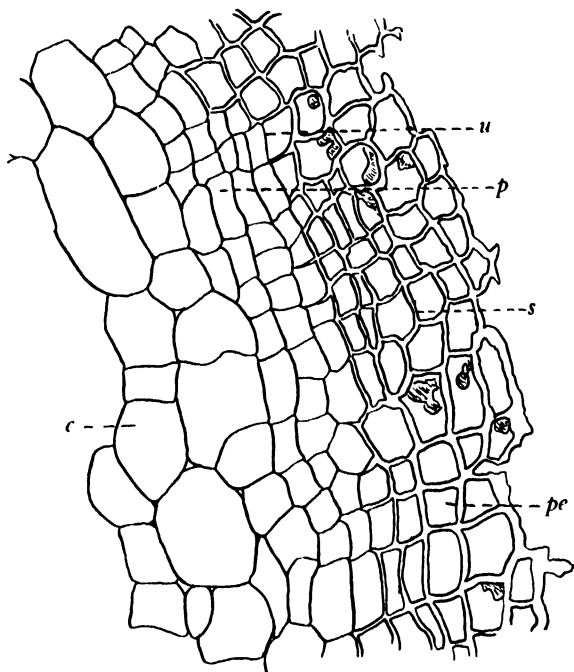


Fig. 17. Transverse section through periderm of root grown on Potterne soil ($\times 266$). *c*, cortex; *p*, phellogen; *pe*, periderm; *s*, suberised wall; *u*, unsuberised wall.

connected with the air content of the soil since, according to Priestley's(2) views, air is necessary for the formation of much suberin. Thus roots from a clay soil with smallest air content have least suberisation, whilst those from the sandy soils with large air content have great deposits of suberin. Potterne soil is also well aerated, and here again the roots show several suberised layers. Roots from calcareous sand and calcareous clay, however, have practically the same amount of suberin deposited in their walls, though the former soil has a relatively higher air content than the calcareous clay.

A second variation is in the number of vessels in different roots. On the whole there are more vessels in roots from the sand and calcareous sand than in the roots from the clay and calcareous clay. Transverse sections of roots taken from sand average 2400 vessels, those from clay 1580; sections of roots grown in calcareous sand have approximately 2000 vessels, while those from calcareous clay have only 1120. This difference is especially marked in the centre of the

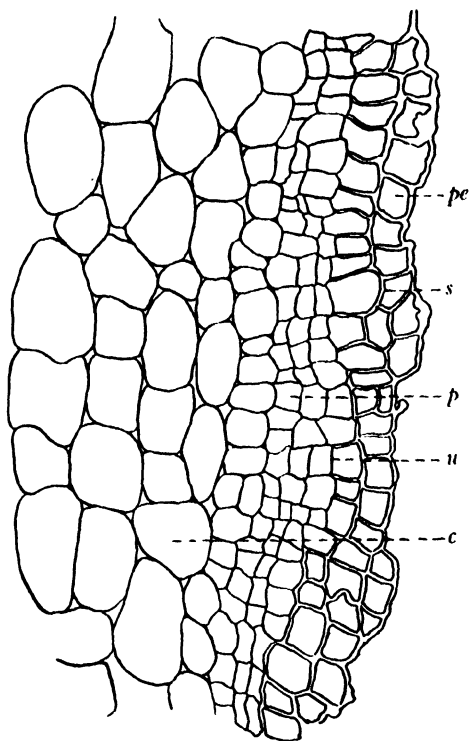


Fig. 18. Transverse section through periderm of root grown on calcareous clay ($\times 266$). *c*, cortex; *p*, phellogen; *pe*, periderm; *s*, suberised wall; *u*, unsuberised wall.

roots, those from the latter soils having fewer and more scattered vessels than the roots from the sandy soils. Likewise the vessels in roots from the two sandy soils show a more evenly distributed radial arrangement, whereas those from the two clay soils are situated in groups separated by wide parenchyma rays. Roots from the Potterne soil form an intermediate stage between the two types. This variation is difficult to explain, though possibly it may be related to the water

supply. The sandy soils are those most quickly subjected to changes in water content, by drying out or by rain. The water content of clay soils is less liable to marked or sudden fluctuations. Therefore the roots growing in sandy soils with variable water content may use more vessels, whereas those in clay soils may need fewer vessels, since their water supply is more constant.

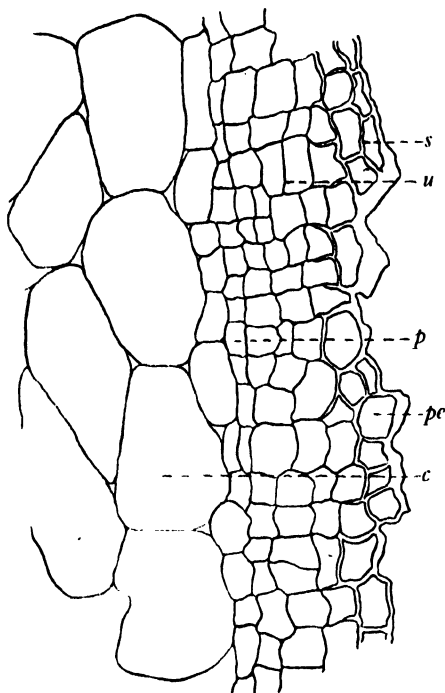


Fig. 19. Transverse section through periderm of root grown on calcareous sand ($\times 266$). *c*, cortex; *p*, phellogen; *pe*, periderm; *s*, suberised wall; *u*, unsuberised wall.

Apart from variations in amount of periderm and number of vessels there appear to be no other anatomical differences in the roots from the five soils. Calcium oxalate occurs fairly equally in all the five types, even in those roots grown in sand, though the soil analyses show that calcium is not present in any determinable quantity in the sand used in the Potterne experiments(3).

VI. CONCLUSIONS

The chief point to be elucidated in the anatomy of the roots of these two species of *Silene* is the possible cause of the bending of the

vessels. It was first suggested that this might be connected with the wrinkling of the surface, both having been produced by contraction. But the vessels bend even in roots which are perfectly smooth, in fact in roots of *S. maritima* which are often quite smooth the vessels appear to be more distorted than in *S. vulgaris*. Though the vessels in longitudinal section appear to have been compressed through contraction, the surrounding cells show no evidence of pressure. The parenchyma cells as described above enlarge as they and the root get older (see Figs. 6, 7 and 8), rather than shrink in volume owing to the withdrawal of sap as described by Thoday(6) to explain the contraction of roots in *Oxalis*, where certain transverse zones of parenchyma cells shrink, causing contraction.

The storage parenchyma cells of *Silene* enlarge as they and the roots mature, possibly owing to the increase in the amount of food reserves in their sap. This would cause an increase in osmotic pressure, and consequently the cells become distended and the vessels more widely separated from one another. It may be that the parenchyma cells enlarge transversely causing considerable pressure on the vessels which become stretched and bent, presuming that such reticulate vessels can stretch, as no evidence is found of broken vessels. That the internal tissues are in a high state of tension and pressure is seen on the emergence of a lateral root. Longitudinal sections through the origin of a lateral root seem to indicate that there is an area of weakness caused by the growth of the branch root. This appears to cause a release of the pressure of the periderm on the surrounding tissues, consequently the tissues, formerly in a state of tension, bulge outwards. This would account for the peculiar arrangement of the vessels at those points, since they have the appearance of being forced outwards by pressure from within.

It has been mentioned that many of the roots are wrinkled at certain points. These bulges, consisting of periderm, phelloderm and cortex, are most often found around the places of external appearance of branch roots. In fact in young roots the wrinkles are found at these points only, for even if the branches are not apparent on the surface, sections show the presence of a new growing root or the remains of a withered one. Some old roots, however, are more wrinkled (Fig. 1). But even so, it does not seem as if contraction can have occurred, since only the older roots or parts of roots of any one plant are wrinkled, the majority of the branches being comparatively smooth, or possessing the small bulges around branches.

VII. SUMMARY

1. This paper describes the anatomical differences between *S. vulgaris* and *S. maritima* and structural variations in *S. vulgaris* due to soil conditions; it does not attempt to explain in detail the various peculiarities, merely to record structural differences.

2. The external characters of the roots are described, the roots of *S. vulgaris* varying in colour and mode of growth according to the type of soil. Wrinkles are present chiefly at the origin of a branch.

3. The primary structure of the roots of both species is diarch. Subsequently secondary growth occurs obliterating this formation.

4. The xylem of the roots of both species is characterised by the large quantity of storage parenchyma, which, as the xylem gets older, enlarges, so that individual vessels are separated from one another by large thin-walled cells.

5. The food reserves in the roots of both species are protein and glucose. Calcium oxalate crystals are present in the parenchymatous tissues.

6. For the roots of both species the older vessels in the centre bend and twist between the large storage cells. The outer younger ones are straighter. Where a lateral root emerges, the vessels are very distorted and have the appearance of being forced outwards by some internal pressure.

7. *S. maritima* differs from *S. vulgaris* in

- (a) possessing a greater number of vessels separated by small, compact thin-walled cells;
- (b) the absence of medullary rays, the xylem forming a continuous ring;
- (c) the presence of definite spring and autumn wood in the more recently formed xylem.

8. Roots of *S. vulgaris* grown on sand, calcareous sand, clay, calcareous clay and Potterne soil vary in the amount of periderm formed and the degree of suberisation. The lighter coloured roots grown in clay have least periderm with very little suberin, the darker roots grown in sand have a large quantity of cork cells which are strongly suberised. Roots from the other soils form intermediate stages between the two extremes.

9. The roots from the five soils also vary in the number and grouping of the vessels. The roots from sandy soils have more vessels with narrow medullary rays than those grown in clay soils.

10. Suggestions of contraction and food storage are considered to explain the bending of the vessels.

In conclusion I should like to express my thanks to Dr W. B. Turrill for his valuable criticism and help, and to Mr E. M. Marsden-Jones for part of the material used.

REFERENCES

- (1) HILL, T. G. and DE FRAINE, E. On the seedling structure of certain *Centrospermae*. *Ann. Bot.* **26**, 178. 1912.
- (2) PRIESTLEY, J. H. and WOFFENDEN, L. Physiological studies in plant anatomy. V. Causal factors in cork formation. *New Phyt.* **21**, 252. 1922.
- (3) MARSDEN-JONES, E. M. and TURRILL, W. B. Report on the Transplant Experiments of the British Ecological Society at Potterne, Wilts. *Journ. Ecol.* **18**, 352. 1930.
- (4) ——— Second Report on the Transplant Experiments of the British Ecological Society at Potterne, Wilts. *Journ. Ecol.* **21**, 268. 1933.
- (5) SOLEREDER, H. *Systematic Anatomy of the Dicotyledons*. Trans. L. A. Boodle and F. E. Fritsch. Oxford, 1908.
- (6) THODAY, D. The contractile roots of *Oxalis incarnata*. *Ann. Bot.* **40**, 571. 1926.

A SEED-PLANT FEATURE OF THE ROOT IN MARATTIACEAE

By R. B. THOMSON

Department of Botany, University of Toronto

(With 2 figures in the text)

TOWARDS the close of the last century an important distinction between the roots of vascular cryptogams and seed plants was pointed out by Sachs, De Bary and Van Tieghem, in their classic contributions to the anatomy of plants. It concerns the orientation of the plane of the lateral root, and while applying to all seed plants is restricted to the forms of vascular cryptogams (ferns both leptosporangiate and eusporangiate) where dichotomous root formation is not involved. Van Tieghem's diagrams illustrating the characteristic conditions are reproduced here (Fig. 1 A and B)—the one at right angles to the main axis and the other parallel to it. Botanists have not since challenged the validity of this distinction.

In 1913 Weiss⁽⁴⁾ recognised the value of its application to the seed ferns (Pteridospermae), and investigated the available material of *Lyginopteris* (*Lyginodendron*) to determine whether the plane of its root was of fern or seed-plant orientation. He found it as in Fig. 1 C, i.e. of seed-plant type, and concluded (p. 7): "*Lyginodendron* would, therefore, in this particular agree with the Flowering Plants rather than with the Ferns."

A few years ago when I was studying the eusporangiate ferns from another standpoint I found an exception to the rule, but did not then fully realise its significance. The eusporangiate ferns, especially the Marattiaceae, have long been considered not only the most primitive ferns but the closest living allies of the seed plants. Usually, however, when a statement to the latter effect has been made it has been qualified by one tending to discount the homology implied by the resemblances. This qualifying statement is that these ferns are homosporous and that being so they cannot be closely related to the seed plants. The argument here, however, is based on a false premise—that the pollen and seed spores differ in size. That they do not was clearly demonstrated by extensive measurements made by the writer in 1927 (2, 3). The discarding of the older view brings many

important aspects of the seed habit into relief and extends the scope of the investigation of seed-plant homologies to a fresh and broader field—the homosporous forms. Since the importance of the resemblances of the Marattiaceae to the seed plants can no longer be discounted we may turn with assurance of proper appreciation to a consideration of the one of these with which we are here concerned—the orientation of the plane of the lateral root in the Marattiaceae.

The assembling of the proper material presented considerable difficulty, both in itself and because most of the roots secured from various sources proved to be polyarch and only diarch roots could be used to determine the orientation plane. However, after assembling and examining many small roots with fine lateral ones attached,

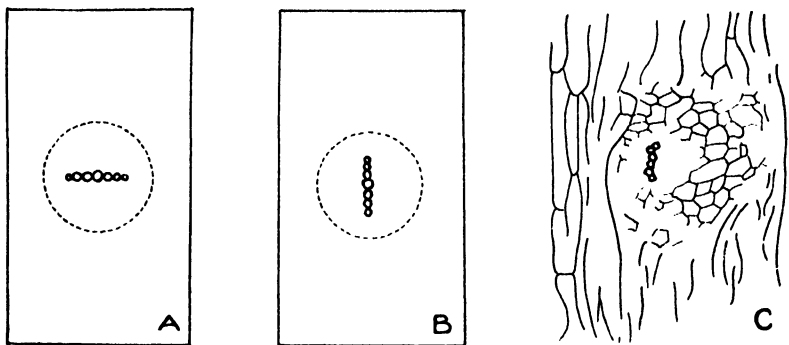


Fig. 1. A, vascular cryptogam; B, seed plant; C, *Lyginopteris*. (A and B after Van Tieghem, C after Weiss.)

enough diarch types were found to demonstrate fundamental differences in orientation. They were not sufficient in number however to determine the relative proportions of the types, and this must be left to those who have access to more abundant material.

In the diagrams (Fig. 2 A–I) the long axis of the main root is vertical and the orientation of the plane of the lateral root with respect to it is indicated by drawings of the wood elements all to the same scale and as they appeared in the sections. In *Marattia alata* (A) the orientation of the two lateral roots examined was longitudinal, as in seed plants. In *Angiopteris evecta* three roots were longitudinal (B) and three obliquely so (C). In *Danaea elliptica* similar conditions were found, one longitudinal (D) and one oblique (E). The one root of *D. jamaicensis* examined was oblique. In the two roots of *Botrychium obliquum* which were studied the plate was transverse (F), and this was true also of a root of *B. ternatum* var.

intermedium and one of *B. virginianum*. Two roots of *Osmunda cinnamomea* were examined to see if there might be any variation

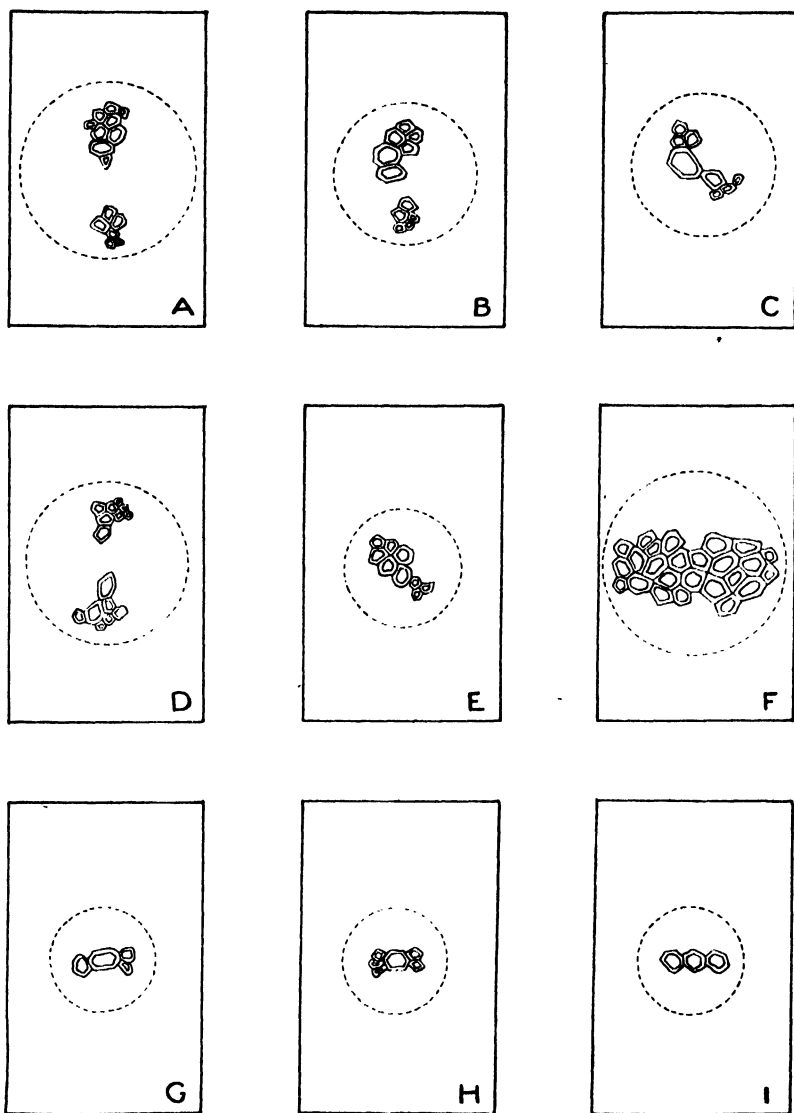


Fig. 2. A, *Marattia alata*; B and C, *Angiopteris evecta*; D and E, *Danaea elliptica*; F, *Botrychium obliquum*; G, *Pilularia globulifera*; H, *Marsilia quadrifolia*; I, *Equisetum arvense*.

in the Osmundaceae, the family of leptosporangiate ferns that are the nearest allies of the Eusporangiatæ. Neither here, however, nor

in the several other homosporous leptosporangiate ferns examined was any deviation from the transverse orientation found. The condition in the heterosporous leptosporangiate ferns was investigated particularly in view of the fact that it would be in these that the most seed-like condition in the ferns should be found if the old view of the necessity of heterospory as a basis of the seed habit should hold. Five diarch roots of *Pilularia globulifera* were sectioned and all showed transverse orientation. The plane of the lateral root in *Marsilia quadrifolia* could be determined definitely in only two cases. It was clearly transverse as in *Pilularia*. All the other lateral roots of *Marsilia* studied were too little developed or too small to have wood elements formed. The indications in these, however, were certainly suggestive of transverse orientation. Three roots of *Equisetum arvense* showed transverse orientation, as in all leptosporangiate ferns.

It is evident that the conditions in the eusporangiate ferns afford a basis for divergent evolution along two lines, the one leading through the Ophioglossaceae and Osmundaceae to the leptosporangiate ferns and the other through the Marattiaceae to the seed plants. The fact that Weiss found that *Lyginopteris*, which has its pollen structure in the form of a synangium as in the Marattiaceous forms, has the seed-plant type of orientation must add weight to the significance of root orientation in modern Marattiaceae. It makes important also an investigation of this condition in the fossil Marattiaceae, whose geological history goes far into the past. Perhaps it will not be out of place to conclude this account with a brief reference to some of the seed-plant features of the Marattiaceae which have been recently brought to light. Land(1) found in *Angiopteris* "a structure comparable with the secondary suspensor of Gymnosperms." The writer in 1927 drew attention to the seed-plant orientation of the embryo in the Marattiaceae, the only vascular cryptogams with such orientation. Recently he has also found in connection with the prolonged enclosure of the embryo in the prothallium that the base of the first leaf becomes haustorial. This makes the term cotyledon, which many botanists have applied to it, perhaps indicative of a closer affinity with the seed plants. With the addition of the new evidence the resemblances between the Marattiaceae and the seed plants become very striking and indicate an intimate connection between the ancestral stock of these two lines.

The *Marattia* material for this investigation came from Johns Hopkins University, Baltimore; the *Angiopteris* from Garfield Park

Conservatory, Chicago, and the Brooklyn Botanic Garden. To the authorities of these institutions I wish to express my indebtedness, and also to Miss M. B. Givens for the preparation of the sections and illustrations.

REFERENCES

- (1) LAND, W. J. G. A suspensor in *Angiopteris*. *Bot. Gaz.* **75**, 421. 1923.
- (2) THOMSON, R. B. Evolution of the seed habit in plants. *Trans. Roy. Soc. Can.* **21**, 229. 1927.
- (3) ——— Heterothally and the seed habit *versus* heterospory. *New Phyt.* **33**, 41. 1934.
- (4) WEISS, F. E. The root-apex and young root of *Lyginodendron*. *Mem. and Proc. Manchester Lit. and Philos. Soc.* **57**, Pt. III. 1912-13.

THE BASIC CHROMOSOME NUMBER OF THE HIGHER PLANTS

By J. H. WANSCHER

Copenhagen

(With 2 figures in the text)

I. INTRODUCTION

AN examination of the reported chromosome counts in the higher plants reveals a large variety of apparent basic numbers. If these numbers, which vary from 3 to the region of 25, are truly "basic," then they would seem in themselves to be of little value in phylogenetic classification. Series are indeed found in various genera, which are of use in elucidating the relationships within these genera, but the very fact that a wide range of numbers may be found within a genus diminishes or vitiates the usefulness of a comparison between genera. The intrageneric ranges overlap, making intergeneric correlation of doubtful value.

The purpose of this paper is, therefore, to define the series and to characterise their origin: to study the origin of chromosome numbers and to find their natural evolutionary sequence.

II. THE STATISTICS OF THE CHROMOSOME NUMBERS

About forty families, all including fifteen or more species, have been selected from Tischler's 1931 list of chromosome numbers. These families, which vary widely in systematic position, are given in Table III, which shows the range of chromosome numbers as given by Tischler and others. In all, the list represents 2563 species from thirty-eight families of the Dicotyledons and 674 species from six families of the Monocotyledons.

The total numbers in the chromosome classes 3 to 24 are given separately for Dicotyledons and Monocotyledons. The frequency curves (Fig. 1) show similarities, the main difference arising from the disproportionately high frequency of the number 7 in the Gramineae. The curve for Dicotyledons seems more regular in its course and will be investigated in greater detail.

A curve of variation of chromosome numbers may indicate either the preference of a special number as being fittest for mechanical or genetical purposes, in which case we should expect a unimodal curve;

or it may show a preferential balance, where the curve would exhibit several modes separated by characteristic distances; or the curve may express the relative ages of the classes, and the trend of their evolution.

The curve of variation of the Dicotyledons is probably determined by many factors, which may act in different degrees. It shows that numbers near 8 and 12, since they are more frequent, are preferred to others. If a special number is favoured, the curve maximum should probably be somewhere between 8 and 12, showing the two

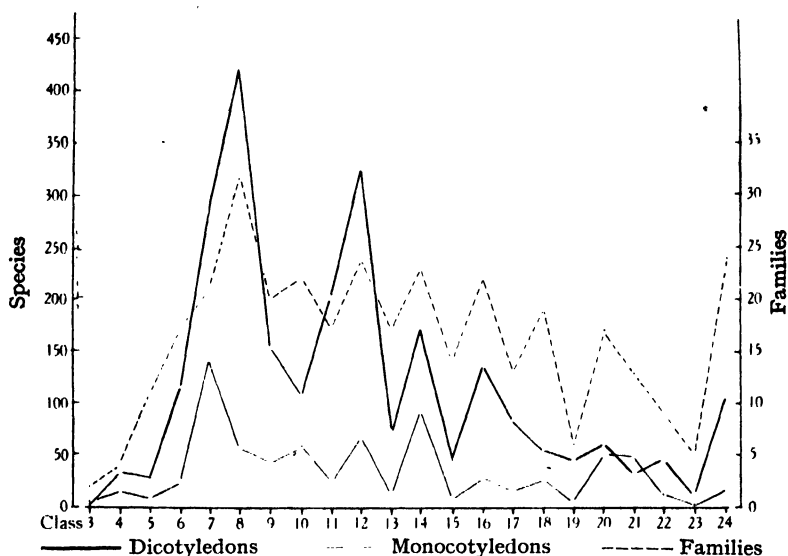


Fig. 1. Diagram showing the variation of the chromosome numbers of forty-four angiospermous families, calculated mainly on Tischler's list (1931). Classes 3-24 inclusive.

maxima of the bimodal curve, the numerical distance being 4. Thus the curve given by Fernandes (1931) also exhibits these characteristic maxima. His curve shows 2220 species in the classes between 3 and 24 and only 194 between 25 and 100. The curves of Fig. 1 show that even numbers are more frequent than odd, and this may be the natural consequence of repeated doubling of lower numbers. Quite definitely, however, the curve of the Dicotyledons expresses the preference for numbers divisible by 4, as shown by the relative maxima at 4-8-12 and 16. Also classes near to these show somewhat high-frequency numbers, and in accordance with the variation of the numbers found in the different families (see Table III) this fact tells

us that new numbers when first established may give rise to new species, forming uniform or other series to nearly the same degree as the mother class from which they have been evolved. The relative sizes of the classes seem therefore to be determined only slightly by such differences, and the curve appears to express the age of the various classes and the trend of evolution.

The different classes show only small differences in their ability to form multiple series. If we compare the frequency numbers of the classes 3-4-5, etc., with those of the classes 6-8-10, etc., their "double derivatives" (Table I), we get by dividing the former by the latter a series of quotients varying from 1/57 to 4/1. The quotient increases from a small fraction and soon reaches a more or less constant value of about 3.

TABLE I

Proportions between the frequencies of species of the classes 3-12 and their "double derivatives" 6-24. The frequency numbers from Table III, Dicotyledons

Class	3	4	5	6	7	8	9	10	11	12
Class	6	8	10	12	14	16	18	20	22	24
Frequencies	2	34	28	115	290	442	155	106	201	327
	115	442	106	327	174	135	56	61	38	107
Quotients	1	1	1	1	3	3	3	2	4	3
	57	13	4	3	2	1	1	1	1	1

These different quotients in some degree express the value of the numbers as basic numbers of multiple series. In accordance with this only a few recently formed multiple series are found with the basic numbers 4, 5 and 6, and none with 3.

The haploid number 3 is extremely rare, being only reported in one species of *Callitriche*, of which genus the other species probably form a series in 5's (5, 10, 19, 20), two *Crepis* species, the others falling into a 4 series, and five species of *Crocus*, which show no polyploidy. Certain species of different Centrosperm and adjoining families show amongst others the numbers 6, 9, 18 and 27, and Tischler (1928) concludes that the primary basic number, although it does not occur, is 3, but this does not seem to be in accordance with the data published by others. The only evidence for this small basic number lies in the numerical distance between 9 and 6, which number has been reported from one species only of Chenopodiaceae among several with 9, and six species of Berberidaceae, where according to Miyaji (1930 b) this number probably originates in a higher

number, 10 (or more probably 8). The remaining members of the 3 series all belong to a 9 series and may also be derived from 8. With this 3 series and other series Tischler combined the families of Centrosperms with the adjoining Berberidaceae (see the genealogical tree of Mez and Ziegenspeck (1926). This is more easily done as these families have about five "series" in common. These several series may, however, be of different origin, and the method is therefore of little value.

According to the normal quotient $3/1$ we may also calculate the fraction of, for instance, the 12 class, to which belong the 6 series: $115/3=38$ species. This would seem to be in accordance with the fact that this 6 series has been found in only two out of the forty-four families investigated, viz. Violaceae with thirty-eight and Plantaginaceae with three species of the 12 class. The remaining part of the 12 class then is of different origin and probably belongs to the 4 series. By far the greatest number of the 14 class seem to belong to a 7 series, and the curve to the right of the 12 class may therefore be only a reflection of the lower part of the curve. The lower part appears to be undisturbed by polyploidy and is therefore of greater interest, and it is the object of the following investigation.

Neglecting hybridisation, except possibly in the 4 series, and also fragmentation and fusion as sources of new numbers (for they probably play only a small part in the formation of the curve), we may divide the chromosome numbers into four, perhaps five, kinds of evolutionary series:

(1) The uniform series: for example 7-7-7-7. This is common in all number classes.

(2) The multiple series: 7-14-21-28, etc., common, in the 7 class and upwards, within one and the same species or genus but rarely as the relation between genera of the same family; that is, intra- and interspecific, rarely intergeneric.

(3) The descending series: 8-7-6-5 and others, usually intergeneric and seldom inter- and intraspecific.

(4) The ascending series: 8-9, etc., as in (3). These two, (3) and (4), arise through loss or gain of chromosomes. As the processes seem sometimes to be reversible, they may be found mixed. They are both aneuploid.

(5) To the fifth class we may perhaps refer the uncertain and irregular *secondary polyploid series*; for example the series 7-14-17 in Pomaceae, 11-19 in Salicaceae, 8-11-14 in Betulaceae.

The uniform series have been mentioned above. They start where

a new balance has been established and do not seem to have any influence on the general variation curve, nor in the lower range (3-17) is the curve disturbed by multiple series. In this part of the curve, as illustrated especially in Fig. 2, the 4 series dominates. For the rest only the multiple 7 series is convincing, while the other series with basic numbers 5 and 6 are only partly represented in the numerical classes 10 and 12, and any calculation as to their significance has been omitted here. The variation curve appears, therefore, to express mainly the evolution of the two aneuploid series 3 and 4, and in some degree to give the course of the descending and ascending processes as such.

Fig. 2 illustrates the variation curve of the chromosome numbers of the classes 3-17 inclusive, drawn on a different scale from that of Fig. 1. The curve shows two peaks at 8 and 12, and the tops of both

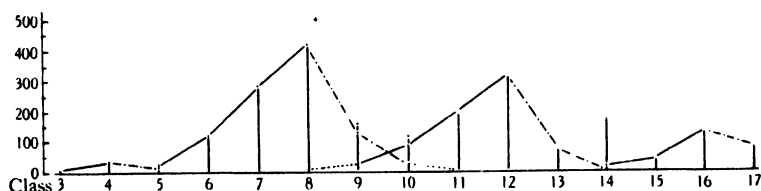


Fig. 2. Diagram showing the course of the descending (full line) and the ascending (discontinuous line) evolutionary series of chromosome numbers in Dicotyledons. Classes 3-17 inclusive. For further explanation see the text.

curves are characteristically skew in the same way. The curve 5-6-7-8-9 and that of 10-11-12-13 show the same course. The falls from 8 to 9 and from 12 to 13 are characteristically much steeper than those from 8 to 7 or from 12 to 11 respectively. This seems to indicate that the ascending process is rarer than the descending.

A tentative analysis into descending and ascending series may be made by assuming that the descending series 8-7-6-5 is little disturbed by chromosome doubling or by chromosome gains. The ratios of numbers in adjacent classes of the series may then be used for calculating numbers to be expected in the 11, 10 and 9 classes by chromosome losses, ultimately from the 12 class. Differences between these numbers and the numbers observed can be ascribed to the ascending series 8-9-10-11, originating by chromosome gain from the 8 class. The calculations have been extended to the 16 and 20 classes, using throughout the ratios of adjacent numbers in the initial descending series 8-7-6-5. The results are shown in Table II

and (in part) in Fig. 2. It is seen that classes 9 and 10, 13 and 14, 17 and 18 are certainly heterogeneous, containing members derived by loss from the next higher and also by gain from the next lower multiple of 4. Observed numbers in the 11 and 19 classes agree fairly well with calculated first terms of descending series from 12 and 20 respectively, suggesting that gains of 3 chromosomes are

TABLE II

The numbers of species are of Dicotyledons in Table III

		Class			
		8	7	6	5
<i>a</i>	Number of species	442	290	115	28
<i>b</i>	Fraction of next higher class	—	.656	.397	.243
		Class			
		12	11	10	9
<i>c</i>	Number of species	327	201	106	155
<i>d</i>	Number calculated from <i>b</i> ("descending series")	—	215	80	26
<i>e</i>	Difference <i>c</i> — <i>d</i> ("ascending series")	—	— 14	26	129
		Class			
		16	15	14	13
<i>f</i>	Number of species	135	46	174	75
<i>g</i>	Number calculated from <i>b</i> ("descending series")	—	89	18	42
<i>h</i>	Difference <i>f</i> — <i>g</i> ("ascending series")	—	— 43	(156)	33
		Class			
		20	19	18	17
<i>i</i>	Number of species	61	45	56	81
<i>j</i>	Number calculated from <i>b</i> ("descending series")	—	40	18	14
<i>k</i>	Difference <i>i</i> — <i>j</i> ("ascending series")	—	5	38	67

infrequent. The high number observed in the 14 class is almost certainly due to doubling of 7 chromosomes, so that the number, 156, ascribed to the ascending series, is much too large.

The results show the possibility of an analysis of the various classes, although it is clear that the assumptions made—that the series 8-7-6-5 is a pure descending series, that the chance of chromosome loss or gain is independent of chromosome number, that doubling is of little importance in lower classes, etc.—are much too simple.

Summarising, the following conclusions may be drawn:

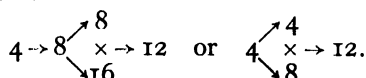
(1) The chromosome numbers of the higher plants originate from numbers belonging to a 4 system.

(2) From this 4 system they develop in different ways. Probably the most frequent course is by loss and gain of chromosomes to form descending and ascending series and from these secondary numbers multiple series may be formed, as from the 4 system itself.

(3) The curves of these two processes, the descending and the ascending, appear to differ; the curve of the latter is the steeper.

(4) The natural succession of the lower chromosome numbers may therefore be given as follows:

For the 4 system:



For the remaining numbers:

in descending series 4-3, 8-7-6-5, 12-11-10-9, 16-15-14;

in ascending series 4-5, 8-9-10, 12-13-14, 16-17.

Numbers such as 9 and 10 come partly from 8 and partly from 12 (according to the curve in Fig. 2). Numbers such as 10, 12 and 14 in increasing degree have their origin in low numbers being also members of multiple series of the fundamental numbers 5, 6 and 7. The diagram shows that the great majority of the 14 class belongs to a 7-14 series. The higher numbers are either multiples of lower basic numbers or of their secondary derivatives.

III. THE BASIC NUMBER OF FORTY-FOUR ANGIOSPERM FAMILIES

The aim of this section is to show that the results of the preceding one hold good in a more detailed investigation of the different families. For many of the families the data to be found in the literature are rather scanty, so far as they concern chromosome numbers and phylogeny, and their basic numbers can therefore only be suggested. The value of the investigation, however, lies in the fact that in the greater number of the families to be considered the cytological and other data point more or less distinctly to basic numbers belonging to or closely related to the 4 series.

The basic number of a given family may be revealed in different ways; through comparison of the shape and size of chromosomes in mitosis and meiosis, and by the study of their behaviour during the reduction divisions, including their primary and secondary associa-

TABLE

The chromosome numbers of

No.	Name	Authority	Class													
			3	4	5	6	7	8	9	10	11	12	13	14		
1	Salicaceae	Tischler, 1927 and 1931	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	Betulaceae	Tischler, 1931	—	—	—	—	—	2	—	—	—	—	—	—	25	—
3	Fagaceae	—	—	—	—	—	—	—	—	—	—	26	—	—	—	—
4	Moraceae	—	—	—	—	—	—	—	—	—	—	4	14	8	—	—
5	Urticaceae	—	—	—	—	—	2	—	—	1	—	4	3	3	—	—
6	Polygonaceae	—	—	—	—	—	—	13	—	26	17	—	—	—	—	—
7	Caryophyllaceae	—	—	—	—	—	—	1	—	1	—	81	—	9	—	—
8	Nymphaeaceae	Langlet and Söderberg	—	—	—	—	—	2	—	1	1	1	—	3	—	—
9	Ranunculaceae	Langlet	—	—	8	8	26	81	—	7	—	6	1	9	—	—
10	Berberidaceae	Miyaji	—	—	—	6	1	2	—	2	—	—	1	11	—	—
11	Cruciferae	Manton	—	—	3	9	55	96	19	5	11	15	1	24	—	—
12	Saxifragaceae	Tischler, 1931	—	—	—	15	21	—	—	—	2	—	24	—	—	—
13	Rosaceae	—	—	—	—	—	91	—	1	—	—	—	—	55	—	—
14	Pomaceae	Moffett, 1931	—	—	—	—	—	—	—	—	—	—	—	—	—	—
15	Amygdalaceae	Tischler, 1931	—	—	—	—	—	28	—	—	—	3	—	—	—	—
16	Leguminosae	—	—	—	—	8	20	74	—	7	3	5	7	1	—	—
17	Oxalidaceae	—	—	—	1	—	14	—	—	—	—	—	—	9	—	—
18	Linaceae	—	—	—	—	—	—	3	15	1	—	1	—	1	—	—
19	Euphorbiaceae	—	—	—	—	3	—	1	6	3	—	—	—	1	—	—
20	Vitaceae	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	Malvaceae	Tischler, 1927 and 1931	—	—	—	—	—	1	—	—	—	—	10	—	—	—
22	Hypericaceae	—	—	—	—	—	1	6	6	—	1	—	—	—	—	—
23	Violaceae	Miyaji	—	—	—	10	1	1	—	27	3	38	3	—	—	—
24	Oenotheraceae	Tischler, 1931	—	—	—	—	42	2	—	—	6	—	—	3	—	—
25	Araliaceae	Wanscher	—	—	—	—	—	—	—	—	—	7	2	—	—	—
26	Cornaceae	Wanscher	—	—	—	—	—	1	2	—	6	—	—	—	—	—
27	Umbelliferae	Wanscher	—	—	—	1	4	19	9	4	66	—	—	—	—	—
28	Bicornes	Hagerup	—	—	—	1	—	2	—	—	—	15	7	—	—	—
29	Primulaceae	Bruun	—	—	—	—	—	9	18	13	74	22	1	1	—	—
30	Oleaceae	Tischler, 1931	—	—	—	—	—	—	—	—	—	—	—	5	—	—
31	Boraginaceae	—	—	—	—	—	—	10	2	—	—	7	—	3	—	—
32	Labiatae	—	—	—	—	—	—	15	9	2	6	2	—	—	—	—
33	Solanaceae	—	—	—	—	—	3	—	6	2	—	75	—	—	—	—
34	Scrophulariaceae	—	—	—	—	37	7	22	18	—	—	12	—	2	—	—
35	Plantaginaceae	—	—	1	2	22	—	—	—	—	—	3	—	—	—	—
36	Dipsaceae	—	—	—	1	—	1	15	27	4	—	—	—	—	—	—
37	Campanulaceae	Tischler, 1927 and 1931	—	—	—	—	5	7	—	—	—	—	1	1	—	—
38	Compositae	—	2	33	13	10	2	8	17	—	5	—	—	—	—	—
39	Gramineae	—	—	—	1	2	89	3	18	10	—	—	2	76	—	—
40	Liliaceae	Fernandes	—	9	1	14	42	33	15	7	10	39	6	6	—	—
41	Amaryllidaceae	—	—	—	2	5	10	5	5	2	3	3	—	3	—	—
42	Iridaceae	Simonet, Mather	5	7	3	2	2	11	2	19	8	19	4	6	—	—
43	Cyperaceae	Tischler, 1931	—	—	1	—	—	2	2	2	—	—	1	—	—	—
44	Orchidaceae	—	—	—	—	—	—	2	1	19	5	6	—	1	—	—
<i>Species:</i> Dicotyledons			2	34	28	115	290	442	155	106	201	327	75	174	—	—
Monocotyledons			5	16	8	23	143	56	43	59	26	67	13	92	—	—
Total species			7	50	36	138	433	498	198	165	227	394	88	266	—	—
<i>Families:</i> Dicotyledons			1	2	6	11	17	26	14	16	13	19	13	19	—	—
Monocotyledons			1	2	5	4	4	6	6	6	4	4	4	5	—	—
Total families			2	4	11	15	21	32	20	22	17	23	17	24	—	—

* Secondary

III

forty-four angiospermous families

Class										Total	Probable basic number	Remarks
15	16	17	18	19	20	21	22	23	24			
—	—	—	—	22	—	—	1	—	1	—	(11) <19	Sec. ass.* in spec. with 19
—	—	—	—	—	—	—	—	—	—	—	8, 7	Sec. ass. in spec. with 14
—	—	—	—	—	—	—	—	—	—	—	4	Sec. ass. and chrom. size
1	5	—	—	—	2	—	—	—	1	—	(4) 8	Sec. ass. in spec. with 13
—	—	—	—	—	—	—	—	—	1	—	8 (7)	
—	—	—	—	—	14	—	15	—	—	—	8	Sec. ass. in spec. with 22 and 10
26	—	2	—	—	2	—	—	—	—	—	4 (8, 12)	
—	—	5	—	—	—	—	—	—	—	—	4 (8)	
1	33	—	—	—	—	8	—	—	4	—	(4) 8	
—	—	—	—	—	—	—	—	—	—	—	4 (8)	
6	34	—	4	1	7	1	3	—	14	—	8	
—	2	—	6	—	—	—	—	—	—	—	8 (7)	
—	—	—	—	—	—	11	—	—	—	—	7	
—	—	43	—	—	—	—	—	—	—	—	7	Sec. ass. in spec. with 17
—	10	—	—	—	—	—	—	—	2	—	8	
—	9	—	—	—	—	1	—	—	6	—	(4) 8	Sec. ass. in spec. with 10, 20, 15 and 24
—	—	—	—	—	—	3	—	—	—	—	7	
5	4	—	1	—	—	—	—	—	—	—	8	
1	1	—	2	—	1	1	—	—	—	—	(8)	
1	1	—	—	22	12	—	—	—	—	—	<19	Sec. ass. in spec. with 19
—	—	—	—	—	3	—	—	—	—	—	8	Sec. ass. in spec. with 13
—	2	—	—	—	3	—	—	—	—	—	8	
—	—	—	3	—	5	—	—	—	11	—	6	Balance at 8?
3	—	—	5	—	—	—	—	—	—	—	(8) 7	
—	—	—	—	—	—	—	—	—	8	—	12	
—	1	—	1	—	—	—	—	—	—	—	8	
—	1	—	—	—	—	—	4	—	7	—	8	Sec. ass. in spec. with 22
—	—	—	1	—	—	—	—	3	7	—	4	Sec. ass. in spec. with 12 and 13
—	1	—	1	—	7	—	12	—	1	—	8	
—	—	—	—	—	—	—	—	6	6	—	(8)	
—	5	—	—	—	—	—	—	—	1	—	(4) 8	
—	11	1	7	—	—	—	—	—	—	—	(4) 8	
—	—	1	6	—	—	1	—	—	30	—	(4) 12	Sec. ass. in spec. with 12 and 24
2	8	3	3	—	4	3	—	4	5	—	(4) 8	
—	—	—	1	—	—	—	—	—	—	—	(4) 6	
—	—	2	7	—	1	—	—	—	1	—	(4) 8	Sec. ass.?
—	3	23	1	—	—	2	—	—	—	—	8	
—	4	1	7	—	2	—	3	—	1	—	4	Sec. ass.
1	—	3	15	—	13	40	2	—	2	—	(8) 7	
4	11	4	5	—	—	1	—	—	5	—	4	Size
1	1	—	—	—	—	—	—	—	1	—	(4)	Size
1	2	7	3	2	11	3	9	1	5	—	4	
1	4	1	1	3	1	4	1	1	1	—	(8)	
—	10	—	3	—	25	—	—	—	4	—	4 (5)	Sec. ass. in spec. with 16 and 20, size
46	135	81	56	45	61	31	38	13	107	2562		
8	28	15	27	5	50	48	12	2	18	764		
54	163	96	83	50	111	79	50	15	125	3326		
9	18	9	16	3	13	9	6	3	18	38		
5	5	4	5	2	4	4	3	2	6	6		
14	23	13	21	5	17	13	9	5	24	44		

association.

tion. The latter is often of special interest; reference may be made to Lawrence (1931*a*), Darlington and Moffett (1930), etc., for further discussion.

In addition to the variation of their chromosome numbers, Table III gives a list of the families investigated and their probable basic numbers. Often two numbers are given, the less certain in brackets and the older to the left. The families are those which contribute 15 or more chromosome numbers to Tischler's list. Together with a few others there are forty-four in all. They follow the usual taxonomical order.

- (1) **Salicaceae** (Blackburn and Harrison, 1924; Håkansson, 1929; Tischler, 1927 and 1931).

The principal number is 19, but the probable secondary association shown by the drawings of cell plates of *Populus* and *Salix* and by the differences in chromosome size may point to a lower primary number. This may be either 11, indicated by the series 22-44 found in *Salix* and the number found in the adjoining order Garryales (*Garrya elliptica*, $n=11$), or 8, the probable ultimate basic number of the following family.

- (2) **Betulaceae** (Helms and Jørgensen, 1925; Woodworth, 1929; Jaretsky, 1930).

The two genera *Carpinus* and *Ostrya* show $n=8$, other genera have 14 or higher numbers. Helms and Jørgensen (1925) find full autosyndesis in the cross *Betula verrucosa*, $n=14 \times B. pubescens$, $n=28$, which points to a primitive set of 7. In *Corylus* and others, Woodworth (1929) reports from 8 to 14 single or compound chromosomes in the metaphases. Three groups of 2 are commonly found and may indicate a double reduplication, 8-11-14, but are more in favour of the basic number 7.

- (3) **Fagaceae** (Jaretsky, 1930; Lawrence, 1931*a*).

The chromosome number of this family is 12 throughout, but different features point to a lower basic number. Thus in his paper (1930) on *Fagales* Jaretsky states that the chromosomes of some *Quercus* species are of different size. On p. 123 he writes about *Q. pontica*: "Ein Vergleich zahlreicher Kernteilungsbilder klärt uns auch bald darüber auf, dass der Chromosomensatz ähnlich wie der von *glandulifera* aus 12 Einheiten besteht, und zwar aus 4 kleinen und 8 mittleren Chromosomen" (see his Figs. 3-8). Clear drawings of metaphase associations of pairs of bivalents also point to the lower basic number and probably to 8 or 4 according to the sizes of the chromosomes and in agreement with the probable basic number of the other family of this order, Betulaceae.

Lawrence (1931*a*) gives 7 as the probable basic number of these families, but I think 8 is in better agreement with the data.

Basic Chromosome Number of the Higher Plants 111

- (4) **Moraceae** and (5) **Urticaceae** (Condit, 1928; Krause, 1931).

The numbers 13 and 14 are common. Probable secondary associations point to lower primary numbers, as also indicated by the numbers 7 and 8 in a few genera of Urticaceae. (See Condit, Pl. 7, figs. 11 and 13 and Krause, Figs. 12 and 15.)

- (6) **Polygonaceae** (Jaretsky, 1928 b).

As basic number of the family Polygonaceae, Jaretsky suggests 11 found in *Rheum*, and that the lower numbers have arisen from this by chromosome elimination: from *Rheum*, 11, to *Rumex*, 10, and *Emex*, 10, and also as far as to *Oxyria*, 7.

Secondary associations of the metaphase chromosomes, as far as may be seen from the figures of Jaretsky in *Rheum* and *Polygonum* species, indicate a lower primary basic number, probably 8, which number is found in some *Rumex* species and in the genus *Fagopyrum*. The number 7 of *Oxyria* has also arisen from 8 rather than from 11.

(Fig. 14 (Jaretsky, 1928 b) shows *Rheum rhaponticum*, $n=22$, 2 groups of three and 5 of two bivalents. Fig. 67, *Polygonum virginianum*, $n=22$, 3 threes and 2 twos. Fig. 83, *P. alpinum*, $n=10$, 1 group of three (?). Fig. 85, *P. sericeum*, $n=10$, 2 twos.)

- (7) **Caryophyllaceae**.

The principal numbers are 12 and 15. The single number 8 of *Corrigiola* and the balance at 15 in *Dianthus* and 14 in *Saponaria* point, like the number 12, to a basic number of the 4 system, 7 being derived from 8.

- (8) **Nymphaeaceae** (Langlet and Söderberg, 1927).

In this family the number 8 of *Nelumbo*, 17 of *Nuphar* and the 7 system of *Nymphaea* seem to point to the primitive base number 8, as 14 is probably derived from 7 ($8-1$) and 17 from 8 ($16+1$). This basic number agrees well with those of the other polycarpic families.

About the different numbers Langlet and Söderberg (1927) write on p. 103: "Die Zahlen sind" (except for *Nymphaea*) "8, 10 und 12, 14, 17, 29, 40, 52. Diese Zahlen bilden mit geringeren Abweichungen eine polyploide Serie mit der Grundzahl 4." The numbers may point to a primitive balance of 4 chromosomes, but as the series has been composed of numbers from different genera, it gives no full evidence of such a 4 system.

- (9) **Ranunculaceae** (Langlet, 1927, 1928, 1932; Böcher, 1932; Moffett, 1932).

114 out of 191 species belong to the numerical series 8-16. 8, therefore, may be taken as the basic number. Of its origin from 4 no traces are left. This small number Langlet calls the "Monoploid-zahl." The next member of the 4 series, 12, has not yet been found as a haploid number. From the cardinal number 8 the other numbers reported have probably segregated by loss or gain of single chromo-

somes: 6, 7 and 9 from 8, and 15 from the double derivative 16, both numbers having been reported by different authors in the same species (*Anemone nemorosa*, Langlet (1932), Böcher (1932) and Moffett (1932) who report 15, 14-16 and 16 respectively). The number 5 of the genus *Paeonia*, which Langlet ascribes to Berberidaceae, probably takes its origin in the primitive number 4.

(10) **Berberidaceae** (Langlet, 1928; Miyaji, 1930 b).

For this family Miyaji points out the numerical evolution from 10 (*Nandina* and *Glaucidium*) through 8 and 7 down to 6. 9 has not been found yet, but there is an evolutionary series 8-7-6 in the genera *Caulophyllum* (8), *Ranzonia* (7), *Epimedium* (6), all belonging to the same karyotype. In the writer's opinion evolution has taken place from 4 to (5), 10 and from 4 to 8, 7, 6 rather than from 10 to 6. Again, 5 may take its origin in a higher number, as indicated by the following three genera all of the same karyotype: *Berberis* (14 or 2×7), *Jeffersonia* (6), *Nandina* (10 or 2×5).

(11) **Cruciferae** (Jaretsky, 1928 a, 1932; Lawrence, 1931 b; Manton, 1932).

The different genera of Cruciferae are often characterised by chromosome numbers in an aneuploid relation to one another, forming descending or ascending series (see p. 105). On the other hand "polyploidy, unlike aneuploidy, has rarely an intergeneric relationship, but is widespread as an interspecific (and intraspecific) relation throughout the family" (Manton, 1932); that is to say that the numbers may form series within the genus and that the different genera are characterised by dissimilar numbers. The following fundamental numbers are found: 5, 6, 7, 8, 9, 11, 13 and 15. By aneuploid changes, i.e. the loss or addition of chromosomes, usually one at a time, these different numbers have probably developed from one primitive set of 8 chromosomes, as Table IV shows. It gives the numbers of genera in the different classes 5-16 incl. (Calculated on the chromosome list of Manton (1932).)

TABLE IV

Genera and species of Cruciferae represented in the classes 5-16

Class	5	6	7	8	9	10	11	12	13	14	15	16
Genera	2	3	24	22	9	2	5	8	1	14	3	14
Species	3	9	55	96	19	5	11	15	1	24	6	34

Of these, class 7 represents fifty-five and class 8 ninety-six species. (In the table they are close together, but the number of species indicates which is the most primitive, viz. 8.) For the *Hesperis* group Jaretsky (1928a) regards 8 as the more primitive from which the other numbers have segregated. He writes (p. 41): "Die 8-Zahl ist die ursprünglichere, aus ihr haben sich die 7-chromosomigen Formen

abgeleitet. Diese sind aber keineswegs monophyletischen Ursprungs, sondern haben sich an verschiedenen Stellen aus 8-chromosomigen Gattungen heraus entwickelt."

That 8 is the primary basic number is also in accordance with the results of Manton. In four diagrams, worked out upon von Hayek's system, she has illustrated the phylogenetic relationship of the different genera of the family. From these it appears (most clearly in Diagram I) that 7 is secondary to 8. In that diagram the groups are given in the following order:

Erysiminae (8) → Cardamininae (8, 16) → Arabidinae (8) $\left\{ \begin{array}{l} \text{Isatidinae (7, 14)} \\ \text{Buniadinae (7, 14)} \end{array} \right.$

Von Hayek makes *Sisymbriinae* (7) as the first tribe, but Manton points out that O. E. Schulz in *Das Pflanzenreich* removes that sub-tribe, so that the artificial assumption of a chromosomal evolution of fundamental numbers 7 to 8 to 7 has thus been avoided (Manton, 1932).

(12) **Saxifragaceae.**

8 is the principal number in *Ribes* and common in *Saxifraga* where it is found together with 7. Other genera show 13 and 18.

(13) **Rosaceae**, (14) **Pomaceae** and (15) **Amygdalaceae** (Darlington and Moffett, 1930; Tischler, 1928).

These three families of Rosales have the basic numbers: Amygdalaceae 8, Rosaceae 7 and Pomaceae 17. The number 7 is likely to be derived from 8, but only a taxonomical investigation will here give any evidence of this evolution. The numerical evolution 8 to 7 is common elsewhere. According to Tischler (1928) 17 is derived from 8, viz. $16 + 1$, but another and much more interesting explanation has been advanced by Darlington and Moffett (1930). They find the primary basic number in two ways: (1) Secondary associations of the metaphase chromosomes in 7 groups when maximal, 3 of three pairs and 4 of two pairs of chromosomes (bivalents); the probable formula being: $3A, 3B, 3C, 2D, 2E, 2F, 2G$ where the letters *A* to *G* represent the original set of 7. (2) "Instead of giving a binomial frequency, or elimination of intermediate numbers, natural seedlings of 'triploid' apples most frequently have numbers of chromosomes approximating to $2n + 7$."

(16) **Leguminosae** (Kawakami, 1930; Kreuter, 1930; Bruun, 1932).

It appears from the statistics that seventy-four out of 124 species of the family have the number 8; 7 follows next with twenty. This number is closely related to 8, as may be seen for example in the genus *Trifolium*, in one section of which the two numbers are mixed without forming any taxonomical series (see Bruun, 1932, p. 203).

Probable secondary associations of the metaphase chromosomes

are seen in the drawings of Kawakami, and Kreuter suggests that plants with higher numbers have the primary basic number 8. A few figures suggest the lower number 4. (See Kawakami (1930): Fig. 1, *Aeschynomene indica*, $n=20$, twos and threes; Fig. 8, *Cassia Leschenaultiana*, $n=24$, twos and threes. Kreuter (1930): Fig. 22, *Amorpha californica*, $n=10$, shows 2 threes and 2 twos; Figs. 24-25, *Amorpha fruticosa*, $n=20$, groups of 6 (?), 3 and 2 bivalents; Fig. 28, *Robinia pseudacacia*, $n=10$, 2 twos; Fig. 36, *Carmichaelia australis*, $n=15$, 1 group of three (?) and 5 twos and 2 single bivalents; Fig. 46, *Astragalus haemosus*, $n=24$, twos and threes?; Figs. 47-50, other *Astragalus* species, $n=8$ (Fig. 48, *A. vulpinus* shows more than 8, 13 (?)), 1-3 pairs of bivalent chromosomes.)

(17) **Oxalidaceae.**

The chromosome numbers of the family include the following: 5, 7, 14 and 21. They point to a 7 series.

(18) **Linaceae** (Emme and Schepeljeva, 1927).

Twelve out of twenty-seven species of the genus *Linum* of this family belong to the 8 series, including the numbers 8, 16 and 15, the latter often found, together with 16, in the same species. Fifteen of the species have the haploid number 9. We do not know whether 8 is derived from 9, but the balance at the number 16 and 15 seems to indicate 8 as the primary base number of the family.

(19) **Euphorbiaceae.**

The number 8 seems to be the basic number of two of the three genera mentioned in Tischler's list (1931): *Mercurialis* (8, 24) and *Daphniphyllum* (16). In the genus *Euphorbia* it is not found. In this genus the following numbers are found: 6, (7), 14, 21, 9, 18, 10, 20 and 15. These series (7, 9, 10) are elsewhere often found together with the 8 series.

(20) **Vitaceae** (Hirayanagi, 1929; Lawrence, 1931 a).

The number 19 is dominant in the family Vitaceae. Secondary associations of bivalent chromosomes in groups of two and three were found by Lawrence in the figures of Hirayanagi (see Lawrence, 1931 a, Table II). Hence he called *Vitis* a secondary polyploid genus. A primary basic number lower than 19 is indicated. The number 16 of *Cissus* points to 8 as basic number.

(21) **Malvaceae** (Tischler, 1927 and 1931; Denham, 1924; Hugh Davie, 1933).

The two families Tiliaceae and Sterculiaceae of the order Columniferae show the chromosome number 8 in one species each. 13 is dominant in Malvaceae, but *Abutilon* shows 8. A slight secondary pairing may be seen in the drawings by Denham of *Gossypium*, $n=13$ (pointed out by Lawrence), and it indicates the lower number

as basic. According to Hugh Davie (1933) this basic number is 7. In *Gossypium herbaceum* he finds maximum secondary pairing in six groups of two bivalents and one single bivalent.

(22) **Hypericaceae** (Winge, 1925; Tischler, 1927 and 1931).

The chromosome numbers 7, 8, 9, 10, 16 and 20 are found in the genus *Hypericum*. Of these 8 is the probable basic number as it, together with 16, is found in eight out of nineteen species, 7 and 9 being probable derivatives. Winge points out a 4 system based upon the series 8-16-20, but as this number 20 has arisen from 10 rather than from 4 (5×4) there is no evidence of this small series left.

(23) **Violaceae** (Miyaji, 1930 a).

According to Miyaji and others 6 is the probable basic number of Violaceae. The 6-12 series is found in different sections, but in the *Nominium* section so far no certain traces of the number 6 have been found. Probably 12 is here the basic number from which other series numbers have segregated: 12-10, 12-13-26-27. 27 has possibly arisen from 9 as so often in other families. If this is so, the basic number lies very near to 8. The "haploid" number 18, the only basis for the "6 series," has been found in two species of the *Nominium* and one species of the *Melanium* sections: (1) *Viola Savatieri*, $2n=36$, which Miyaji calls a probable species hybrid between *V. mandschurica*, $n=24$, and a species with 12; (2) *V. kisoana*, $2n=36$, probably a triploid species also, and (3) in *V. Kitaibeliana* which in addition shows the haploid numbers 7 and 8, suggesting that the number 18 here is composed of two sets of 9. Further, the number 17 found in three other species of the same subsection Tricolores points to a primitive balance of 8. A chromosome balance of this numerical order is found elsewhere in Parietales: Cistaceae (8-16, 9), Passifloraceae (9), Caricaceae (9) and Cucurbitaceae (McKay, 1931) (7 [1] 10 [3] 11 [8] 12 [12] 13 [2] 16 [4] 20 [7] 21 [1] 24 [2]). (Numbers of species in [].) In short: 6 is the probable basic number in parts of the family, but in the *Melanium* and maybe also in the *Nominium* sections traces of the old balance at 8 are still found.

(24) **Oenotheraceae** (Håkansson, 1931).

The greater part of the family Oenotheraceae has the base number 7 (found in thirteen out of nineteen genera). The number 8 plays a smaller rôle being found in two or three only, and its common derivative, 9, in three genera. As primary basic number we may suggest 8.

(25) **Araliaceae**, (26) **Cornaceae** and (27) **Umbelliferae** (Wanscher, 1931, 1932, 1933 a, b).

The three families of the order Umbelliferae have different numbers. Araliaceae show 12 (13), Cornaceae 8, 9 and 11, and

Umbelliferae 8 and 11 besides numbers varying between 6 and 48 haploid. The number 8 is probably the primary in the last family for the following reasons (for specified description see Wanscher, 1933 a and b):

(1) Most of the primitive genera investigated have the basic number 8; *Hydrocotyle* (?), *Bowlesia*, *Azorella*, *Sanicula*, *Hacquetia*, *Eryngium* from the two first subfamilies and from the third, Apioideae, the following: *Anthriscus* 6, 7, 8, 9, *Scandix*, *Torilis* and *Bupleurum*, 7, 8.

(2) *Bowlesia* showed as far as could be stated in the haploid set of 8 (embryo sac) four types of chromosomes, two of each, indicating the still lower 4.

(3) Types with higher numbers than 8 sometimes show secondary association pointing to the lower number, *Molopospermum* and *Hydrocotyle* with 22 and 24, 48 respectively (see Wanscher, 1933 a).

Due to the high basic number 12 Araliaceae cannot be ancestral to Umbelliferae as usually maintained.

(28) **Bicornes (Ericaceae)** (Hagerup, 1927, 1928).

The different families of the order Bicornes show according to Hagerup the following numbers: Pyrolaceae, 23; Rhodoraceae, (6), 12, 13 and 24; Empetraceae, 13, 26; Ericaceae, 8, 12, 13, 18, 24, 26 and 48; Vacciniaceae, 12, 24, 36 and 30; Epacridaceae, 13; Clethraceae, 8, 16; Diapensaceae, 6. Of the numbers Hagerup writes: "We thus see that the numbers fall into (1) a very good 6 series, 6, 12, 18, 24, 30, 36 and 48; (2) a small 13 series, 13 and 26; and (3) an 8 series, 8 and 16." From these numbers he concludes that 6 is the fundamental number of the order. But as the 6 series has been composed of numbers from different families it gives very little evidence for such a basic number.

The "basic number" itself is found only in one species, *Diapensia lapponica*. It is given by Hagerup also for *Phyllodoce coerulea*, but his drawings (Figs. 11 and 13) show 10-12 (diakinesis) and 12 respectively (second metaphase: one cell with 6 and one with 12). I have been able to check this number 12 from Hagerup's own preparations, which he has kindly put at my disposal for investigation.

The other member of a special 6 series, 18, is found in one species of Ericaceae where 8 is reported from *Calluna* and 12 from many other genera. There is no evidence whatever of this number being composed by 3 sets of 6. It has rather arisen from 9 (8+1). The most common number in the order is 12 (13) which together with 8 is a member of the 4 system strongly indicated by secondary association in *Empetrum nigrum*, $n=13$, showing maximum association at 4-3-3-3. Also grouping found in *Erica cinerea*, $n=12$, *Ledum groenlandicum*, $n=13$, indicates the low number 4. (For further description of this material, kindly put at my disposal by Dr O. Hagerup, see Wanscher (1933 b).)

(29) **Primulaceae** (Bruun, 1932).

According to the opinion of Bruun "very likely the development of *Primula* proper (excl. *Auricula* and *Verticillatae*) started in conjunction with the origin of this number 11," to be found in 46 per cent. of the total number of species. "This basic number (11) is changed unexpectedly in three subsections of *Primula*, viz. (1) from 11 to 12 and 13 in subsection *Geranioides* of *Cortusioides*; (2) from 11 to 10, 9 and 8 in different subsections of *Farinosae*."

From my point of view the whole subgenus *Primula* is of the greatest interest as containing sections with the "archaic" number 8 and its common derivative 9. It consists of four sections:

- Sect. *Soulei* (1 spec.) with the chromosome number 8.
- „ *Farinosae* subsection *Stenocalyces* (5 spec.) 8.
- „ *Farinosae* subsection *Eufarinosae* (13 spec.) 9 series.
- „ *Farinosae* subsection *Sibiricae* (6 spec.) 9, 10, 11.
- „ *Farinosae* subsection *Glabrae* (2 spec.) 8.
- „ *Yunnanensis* (1 spec.) 11.
- „ *Minutissimae* (1 spec.) 11.

Of these groups those with 8 are specialised alpine types and the group with the 9 series, *Eufarinosae*, is a widespread and taxonomically old and isolated group. Possibly these groups with 8 and 9 separated before the stabilisation of the number 11, just as Bruun explains the isolation of the older ramifications *Auricula* and *Verticillatae*. In the drawings of the meiosis of *P. burmanica*, Bruun shows differences in shape and size of the chromosomes which possibly indicate the origin of the number 11. His Fig. 31 shows in prometaphase and beginning of anaphase 4 chromosomes with two terminal chiasmata and 7 ($8 \div 1$?) with a single terminal chiasma.

(30) **Oleaceae.**

As far as it can be seen from the chromosome numbers in Tischler's list the basic number lies near 8 in this family. *Forsythia* has 14, probably $7+7$, and *Syringa* has 24, 3×8 or 2×12 .

(31) **Boraginaceae.**

The number 8 is found in ten out of fifteen genera of *Boraginaceae*, and the number 12 in three to four of these. Only in one genus each, 7 and 9 have been found. There seems to exist therefore a primitive balance of 4 chromosomes.

(32) **Labiatae.**

In the family *Labiatae* 8 has been found in five out of nine genera, the number 9 in two and 12 in two also. Probably in this family we have a 4 system also.

(33) **Solanaceae** (Belling and Blakeslee, 1922 and 1926; Kostoff, 1926; Tischler, 1928; Jørgensen, 1928; Bleier, 1930; Meurman, 1932; Müntzing, 1932; Sansome and Philp, 1932).

The number 6 has often been referred to as the basic number of Solanaceae. The evidence for this assumption lies in the first and third member of the 6 series 6-12-18-24-36-48 (see Tischler, 1928). The number 6 has been reported by Kostoff (1926) in varieties of *Capsicum annuum*, the normal number of which by other authors is given as 12. Nor has the number been revealed indirectly. Haploid *Daturas* (Belling and Blakeslee, 1922 and 1926) show random assortment of the chromosomes, which are unpaired. About 88 per cent. of the pollen grains abort. The next member of the "6" series is the haploid number 18 of triploid plants with 36 chromosomes in the soma. In meiosis they form trisomes or bivalents and univalents. They show no autosyndesis (Jørgensen, 1928; Müntzing, 1932). The 2-1 disjunction of each trivalent leads to a maximum of gametes with 18 (Sansome and Philp, 1932). The remaining members of the series all belong to a 12 series which does not seem to have this low origin, viz. 6.

We do not know the origin of 12, but there seem to be a few traces left of a primitive balance of 4 and 8. In addition to the number 12 itself, the number 7 of *Petunia*, 9 of six species of *Nicotiana* and 16-32 of another species of this genus all point to this 4 series. Also the sizes of the chromosomes in *Datura* ($n=12$) measured by Belling and Blakeslee (1926) point to this series. They give six size classes although three seem more in accordance with the data (see Table V).

TABLE V

Chromosomes of haploid and triploid Datura
(after Belling and Blakeslee)

Classes	No. of chromosomes	Length		Classes proposed by author
		Haploid	Triploid	
<i>I</i> .	1	54	55	1 long (54-55)
<i>l</i>	4	36	41	
<i>M</i>	3	31	30	7 medium (30-41)
<i>m</i>	2	21	23	
<i>S</i>	1	16	21	4 short (13-23)
<i>s</i>	1	13	19	

We thus obtain 8 long (long and medium) and 4 short chromosomes in the haploid set.

Further secondary associations reported by Meurman and seen in Bleier's figures of potato varieties point to a low origin of the number 12. Müntzing (1932) gives material which seems to be strongly in favour of the 4 series, but this view is not held by the author, who concludes that the basic number of *Solanum tuberosum* is 6 as

suggested by others. In a diploid strain he finds that 38 per cent. of the chromosomes in second metaphase plates are found in groups of two bivalents (4 chromosomes), in a triploid 21 per cent. in groups of three, and in a tetraploid 13 per cent. in groups of four (see Table VI). From this he concludes that groups of two, three and

TABLE VI

Percentages of chromosomes found singly or in groups of two to five or higher secondary associations in diploid, triploid and tetraploid potato according to Müntzing (1932)

Strain	Total	Single %	Groups of				Higher %
			2 %	3 %	4 %	5 %	
Diploid	552	55	38	4.5	1.5	0.9	—
<hr/>							
Triploid	696	49	27	21	3.2	0.8	
<hr/>							
Tetraploid	584	32	20	15	13	3.9	circa 17.0
<hr/>							
						20	

four bivalents are characteristic of diploids, triploids and tetraploids respectively; hence he concludes that 6 is the basic number. But he fails to see that groups of higher order are found in these three strains. Thus no less than 4.5 per cent. of the chromosomes of the diploid are found within the groups of three.

Assuming the basic number to be either 6 or 4 we may write the formulas as follows:

$$\begin{array}{lcl}
 & A A & \\
 & B B & \\
 & C C & \\
 (1) \quad D D & \text{or } (2) & A A A \\
 & & B B B \\
 & E E & C C C \\
 & F F & D D D
 \end{array}$$

Formula (1) allows a maximum association of 6 groups of two gemini and none of higher groups. Formula (2) allows a maximum association of four groups of three gemini, and four groups of two may be found.

Of 46 investigated cell plates (collected in a table (p. 225) by Müntzing, 1932) one only is strictly against formula (2) having five groups of two gemini. It is thus in favour of formula (1) also. Seven plates are strongly in favour of formula (2) and at the same time against (1) showing higher associations than groups of two. They show one or two associations of three gemini each. Thirty-five plates with 1-4

pairs of gemini are indifferent. Three cells with associations of 4 and 5 gemini are, although uncertain, in favour of formula (2). Thus, I think, the chromosome number 12 has arisen rather from 4 than from 6.

(34) **Scrophulariaceae** (Bruun, 1932).

In Scrophulariaceae different genera show different basic numbers: *Linaria*, 6; *Cymbalaria*, 7; *Antirrhinum* and *Pentstemon*, 8; *Nemesia*, 9; other genera show more variation in their numbers. The genus *Verbascum* thus shows 15 (2), 16 (7), 18 (2), 24 (1), 32 (1), and has 8 as the probable basic number. The genus *Veronica* has 7 (1), 8 (1), 9 (1), 12 (1), 16 (3), 17 (1), 20 (2), 24 (1). Also in this genus we find an 8 series, from which the other numbers have separated by aneuploid evolution. In *Veronica* we find the descending series 8-7-(6) (indicated by 12). The basic numbers of the three first-mentioned genera form a similar series. As 8 is thus found to be the basic number of four genera against one with 9 I conclude that 8 is primary and therefore not a member of the two descending series of the genus *Veronica* and of the genera mentioned above. Such an increase of the number is found elsewhere in the family, e.g. 16-17 in *Veronica*.

(35) **Plantaginaceae**.

Most of the species of this family, genus *Plantago*, have 6 as basic number. The number 4 of *P. ovata* is either a relic of the old number or it is of secondary origin, from 5 and this again from 6 or *vice versa*.

(36) **Dipsaceae** (Risse, 1928).

The different genera show either 8 or 9 or both together. In his drawings of first metaphase of *Cephalaria leucantha*, $n=8$, and according to the author there are distinctly five single gemini, one normally secondarily associated pair of two gemini and a large 8-shaped double chromosome(?). Such configurations may account for his finding 8 chromosomes throughout the family, even where other authors find 9. The association points to a lower basic number than 8.

(37) **Campanulaceae** (Tischler, 1927 and 1931).

In five out of eight genera of Campanulaceae the numbers 8 and 16 are found, in *Campanula* together with the secondary derivative 17 and in *Lobelia* together with 7 and 9, the former being the fundamental number of the series 7-14-21. We may suggest 8 as the primary basic number of the whole family.

There are, as far as is known, no traces left of the primitive balance at 4.

(38) **Compositae** (Tischler, 1927 and 1931; Lawrence, 1931 a).

Compositae is one of the very few families where the "archaic" number 4 is still in existence and plays an important rôle. Statistically it reaches the maximum with thirty-three species (Tischler's

list (1931)). It is found in six genera: *Crepis* (32 specimens), *Picris* (2), *Urospermum* (1), *Hypochaeris* (1), *Leontodon* (1) and *Scorzonera* (1), all of them from the subfamily Liguliflorae (Tischler, 1927 and 1931).

The next basic number met with is 5. It is found in 9 genera, including *Senecio* which is not taken into the statistics in Table III, and in five of these together with 4 or 8 which are here in the majority; hence it is probably of secondary origin from 4. In *Senecio* and other genera it is a fundamental number.

The very common number 9 may owe its origin to 8 as in *Dahlia*. In *Dahlia Merkii*, $n=18$, Lawrence (1931a) finds secondary association of the metaphase chromosomes in the maximum of 2 three and 6 two, which points to 8 as basic number as in other *Dahlia* species, and in *Hidalgoa Wercklii* he reports groups of two and three also; here the grouping indicates a lower number than 8.

(39) **Gramineae** (Darlington, 1933).

In Gramineae more than two-thirds of the species given by Tischler (1931) belong to the 7 series: 205 out of 275. Naturally, therefore, we conclude that 7 is now the basic number of the family. Whether it takes its origin in the higher number 8 we do not know, the higher fundamental numbers 9 and 10 found at different places of the system and the number 8 itself found in *Secale* species side by side with 7 possibly indicate this higher primitive basic number. According to Darlington (1933) this eighth chromosome behaves like a fragment, and the number 8 is therefore secondary to 7.

(40) **Liliaceae** (Fernandes, 1931; Levan, 1932).

In simple statistics the number 7 reaches the top of the curve in this family also, but if we take the whole 7 series (7-14-21) against the 4 series (4-8-12-16-24) we get 48 and 94 species respectively. Thus the statistics point to 4 as basic number.

Cytological data also indicate this primitive number. Fernandes reports, in ten species of the genus *Aloë*, $n=7$, four long and three very short chromosomes (fragments?). From *Allium macranthum* Levan reports: (1) 6 long chromosomes with median constriction, (2) 4 medium with subterminal constriction, and (3) 4 short with median submedian constriction.

(41) **Amarylloidaceae** (Fernandes, 1931).

Statistically no special number is marked as basic number of the family. Class 7 has ten species, classes 6, 8 and 9 have five species each. In his figures (15, 18, 19, 21, 22, 23 and 24) Fernandes (1931) shows in *Narcissus* species 4 big and 2-3 small chromosomes as in the *Aloë* species mentioned above. The small chromosomes are not as minute as in that genus. Probably 4 has played its part here too.

(42) **Iridaceae** (Simonet, 1932; Mather, 1932).

The two main genera *Crocus* and *Iris* of this family both show a great variation in chromosome numbers, the former from 3 to about 23 haploid and the latter from 8 to about 54. The many different numbers have probably arisen through hybridisation, polyploidy and fragmentation (Mather, 1932). Statistically no basic number is indicated either in *Crocus* or in *Iris*; only one section of the latter genus shows a definite fundamental number: 4 of the series 8-12-16-20-24. Simonet calls it the "nombre chromosomique des *Iris*." In different hybrids and in different species of the section *Pogoniris*, he finds in the meiotic divisions 16_{II} and 12_I , and these numbers may point to the primitive number.

(43) **Cyperaceae** (Heilborn, 1924, 1928; Tischler, 1927 and 1931).

The chromosome numbers of Cyperaceae vary from 5 to 56, more than two-thirds belonging to classes higher than 25 and therefore not represented in the statistics. The numbers form an aneuploid series without any polyploids. It is therefore impossible from the numbers alone to draw any conclusions as to the original fundamental number. According to Heilborn (1924) evolution in *Carex* has taken place from species with low numbers (9 is the lowest found), these plants being adapted to dry localities, to species with higher numbers (maximum 56) adapted to wet localities. Possibly the genus thus started from 4 or 8, the latter being found in *Scirpus* and *Heleocharis* (one species each); 16 is reported in one species of *Heleocharis* and in *Dulichium*.

(44) **Orchidaceae** (Hoffmann, 1930).

The number 4 is probably the primitive basic number of Orchidaceae, as a great number of the species investigated belong to the 4 series, 8-12-16-24 (twenty-two species). The series is composed of numbers from different genera. Most of the other genera and species of the family belong to a 10-20 series, of which probably 5 is the fundamental number.

Cytological data also point to the low basic number. Thus Hoffmann reports from first meiotic metaphase of *Paphiopedilum Leeatum* (*P. insigne* \times *Spicerianum*), $n=12$, 4 large, 4 medium and 4 small chromosomes (see his Fig. 6). Secondary associations to be recognised in his different figures also point to the low basic number.

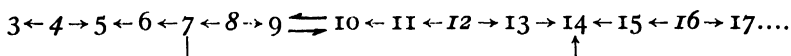
[Fig. 4, *Paphiopedilum insigne*, $n=16$ (?), early anaphase: twos and threes. Fig. 31, *Epidendron nocturnum*, $n=20$, first metaphase: 3 twos and 1 group of 3. Fig. 32, *Epidendron raniferum*, $n=20$, early anaphase: 6 twos. Fig. 43, *Dendrobium infundibulum*, $n=20$, first metaphase: groups of 2, 3 and 4. Fig. 45, *Polystachya polychaete*, $n=20$, early anaphase shows 5 groups of 3 and five single chromosomes, which seems to indicate maximum association: 5 groups of 4, and 5 as basic number of the 10-20 series.]

Summing up. For families with higher basic numbers, such as Salicaceae and Vitaceae, the observations of secondary association suggest a lower original basic number. The remaining families all seem to have basic numbers belonging to the 4 system, either 8 or 4 or 12, or else numbers closely related to it, e.g. 7, and for two families 6. No family shows 3 or 5 as a basic number.

IV. SUMMARY

The frequency curve of the chromosome numbers shows that the great majority of species have numbers lying between 3 and 24, and within the limits of these two classes the curve shows maxima at 8 and 12, the numerical distance being 4 (see Fig. 2).

The numbers as they appear in the different families may be divided into four different evolutionary series: (1) the uniform series, for instance, 7-7-7-7; (2) the multiple series, 7-14-21, etc.; (3) the descending series, 8-7-6-5; (4) the ascending series, 8-9-10. As (1) and (2) do not seem to have any real influence on the relative sizes of the number classes in the lower part of the curve as shown in Fig. 1, the curve expresses the relative age of the classes in evolution. It therefore expresses the course of (3) and (4) and shows that they start at numbers of the 4 series:



The arrows show the direction of evolution. The members of the 4 series are proper to the other numbers.

In forty-four selected families (contributing more than fifteen species each to Tischler's list (1931)) I have tried to reveal and characterise the general rôle of this primary set of four. Table III gives the full list of these families together with their chromosome numbers and their probable primary basic numbers as they are stated in the literature quoted. In the second part of this paper these families are examined separately. In thirty-seven of the forty-four families there is definite evidence of a 4 system, or some indication of it. In five families we may trace the basic number to the number 7, which is probably to be reckoned as derived from 8. Two families have the basic number 6.

I wish to express my gratitude to Professor Ø. Winge for his general interest and for his help and advice.

REFERENCES

- BANGHAM, W. The chromosomes of some species of the genus *Philadelphus*. *Journ. Arnold Arb.* **10**, 167. 1929.
- BELLING, J. and BLAKESLEE, A. F. The assortment of chromosomes in haploid *Daturas*. *La Cellule*, **37**, 355-61. 1926.
- BLACKBURN, K. R. and HARRISON, J. W. HESLOP. A preliminary account of the chromosomes and chromosome behaviour in the Salicaceae. *Ann. Bot.* **38**, 361-78. 1924.
- BLAKESLEE, A. F., BELLING, J., FARNHAM, M. E. and BERGNER, A. D. A haploid mutation in Jimson Weed. *Science*, **55**, 646. 1922.
- BLEIER, HUBERT. Untersuchungen über die Sterilität der Kartoffel. *Arch. f. Pflanzenbau*, **5**, 545-60. 1930.
- BÖCHER, T. W. Beiträge zur Zytologie der Gattung *Anemone*. *Botanisk Tidsskrift*, **42**, 183-206. 1932.
- BRUNN, H. G. Cytological studies in *Primula* with special reference to the relation between the karyology and taxonomy of the genus. *Symbolae Botanicae Upsaliensis*, **1**, 1-339. 1932.
- CONDIT, I. J. Cytological and morphological studies in the genus *Ficus*. I. Chromosome number and morphology in seven species. *Univ. Calif. Publ. Bot.* **11**, 233-44. 1928.
- DARLINGTON, C. D. *Recent Advances in Cytology*. Pp. 559. London. 1932.
- The origin and behaviour of chiasmata. VIII. *Secale cereale* ($n=8$). *Cytologia*, **4**, 444-52. 1933.
- DARLINGTON, C. D. and MOFFETT, A. A. Primary and secondary balance in *Pyrus*. *Journ. Gen.* **22**, 129-63. 1930.
- DAVIE, J. HUGH. Cytological studies in the Malvaceae and certain related families. *Journ. Gen.* **28**, 33-67. 1933.
- DENHAM, H. J. The cytology of the cotton plant. *Ann. Bot.* **38**, 407-32. 1924.
- EMME, H. and SCHEPELJEVA, H. Versuch einer karyologischen Artanalyse von *Linum usitatissimum* L. *Bull. Applied Bot. Gen. and Plant Breeding*, **17**, 265-71. 1927.
- FERNANDES, A. *Estudos nos cromosomas das Liliáceas e Amarilidáceas*. Pp. 122. Coimbra. 1931.
- HAGERUP, O. *Empetrum Hermaphroditum* (Lge) Hagerup. A new tetraploid species. *Dansk Bot. Arkiv*, **5**, No. 2. 1927.
- Morphological and cytological studies of *Bicornes*. *Dansk Bot. Arkiv*, **6**, No. 1. 1928.
- HÅKANSSON, ARTUR. Die Chromosomen in der Kreuzung *Salix viminalis* × *Capraea* von Heribert Nilsson. *Hereditas*, **13**, 1-52. 1929.
- Chromosomenverkettung bei *Godetia* und *Clarkia*. *Ber. d. Deut. bot. Gesell.* **49**, 228-34. 1931.
- HAYEK, A. VON. Entwurf eines Crusifere-system auf phylogenetische Grundlage. *Beih. z. bot. Zentr.* **27**. 1911.
- HEILBORN, O. Chromosome numbers and dimensions, species formation and phylogeny in the genus *Carex*. *Hereditas*, **5**, 129-216. 1924.
- Chromosome studies in Cyperaceae. *Hereditas*, **11**, 182-92. 1928.
- HELMs, ANNA and JØRGENSEN, C. A. Birkene paa Maglemose. Magle-Mose i Grib Skov, vol. 1, VII. København, pp. 181-258. 1925.
- HIRAYANAGI, H. Pollen mother cells of the vine. *Mem. Coll. Sci. Kyoto*, **B**, **4** (3). 1929.
- HOFFMANN, K. M. Beiträge zur Zytologie der Orchidaceen. *Planta*, **10**, 523-95. 1930.
- JARETZKY, J. Untersuchungen über Chromosomen und Phylogenie bei einigen Cruciferen. *Jahrb. f. wiss. Bot.* **68**, 1-45. 1928 a.
- Histologische und karyologische Studien an Polygonaceen. *Jahrb. f. wiss. Bot.* **69**, 357-490. 1928 b.
- Zur Zytologie der *Fagales*. *Planta*, **10**, 120-37. 1930.

Basic Chromosome Number of the Higher Plants 125

- JARETZKY, J. Beziehungen zwischen Chromosomenzahl und Systematik bei den Cruciferen. *Jahrb. f. wiss. Bot.* **76**, 485-527. 1932.
- JØRGENSEN, C. A. The experimental formation of heteroploid plants in the genus *Solanum*. *Journ. Gen.* **19**, 133-211. 1928.
- KAWAKAMI, J. Chromosome numbers of Leguminosae. *Bot. Mag.* **44**, 319-28. 1930.
- KOSO-POLJANSKI. *Caucalis leptophylla*. *Bull. Soc. imp. nat. Moscou*, **27**, 151. 1925.
- KOSTOFF, D. Die Bildung der Pollenkörner bei einigen Varietäten von *Capsicum annuum*. *Ann. Univ. Sofia Fac. Agron.* **4**, 101-21. 1926.
- KRAUSE, OTTO. Zytologische Studien bei den Urticales unter besonderer Berücksichtigung der Gattung *Dorstenia*. Diss. Kiel. 1931.
- KREUTER, E. Beitrag zur karyologisch-systematischen Studien an Galegeen. *Planta*, **11**, 1-44. 1930.
- LANGLET, O. Beiträge zur Zytologie der Ranunculaceen. *Svensk Bot. Tidskrift*, **21**, 1-27. 1927.
- Einige Beobachtungen über die Zytologie der Berberidaceen. *Svensk Bot. Tidskrift*, **22**, 169-84. 1928.
- Über Chromosomenverhältnisse und Systematik der Ranunculaceae. *Svensk Bot. Tidskrift*, **26**, 381-400. 1932.
- LANGLET, O. and SÖDERBERG, E. Über die Chromosomenzahlen einiger Nymphaeaceen. *Acta Horti Berg.* **9**, 85-104. 1927.
- LAWRENCE, W. J. C. The secondary association of chromosomes. *Cytologia*, **2**, 352-84. 1931 a.
- The chromosome constitution of *Cardamine Pratensis* and *Verbascum Phoeniceum*. *Genetica*, **13**, 183-208. 1931 b.
- LEVAN, ALBERT. Cytological studies in *Allium*. II. *Hereditas*, **16**, 257-94. 1932.
- McKAY, J. W. Chromosome studies in the Cucurbitaceae. *Univ. Calif. Publ. in Bot.* **16**, 339-50. 1931.
- MANTON, IRENE. Introduction to the general cytology of the Cruciferae. *Ann. Bot.* **46**, 509-56. 1932.
- MATHER, K. Chromosome variation in *Crocus*. I. *Journ. Gen.* **26**, 129-42. 1932.
- MEURMAN, O. and RANCKEN, G. Untersuchungen über die Chromosomenverhältnisse bei kultivierten Kartoffelsorten (*Solanum tuberosum* L.). *Comment. Biol. (Soc. Sci. Fennica)*, **3**, 1-27. 1932.
- MEZ, K. and ZIEGENSPECK, H. *Königsberger serodiagnostischer Stammbaum*. Königsberg. 1926.
- MIYAJI, Y. Betrachtungen über die Chromosomenzahlen von *Viola*, *Violaceae* und verwandten Familien. *Planta*, **11**, 631-49. 1930 a.
- Beiträge zur Chromosomenphylogenie der Berberidaceen. *Planta*, **11**, 650-9. 1930 b.
- MOFFETT, A. A. The chromosome constitution of the Pomoideae. *Proc. Roy. Soc. B*, **108**, 423-46. 1931.
- Chromosome studies in *Anemone*. I. A new type of chiasma behaviour. *Cytologia*, **4**, 26-37. 1932.
- MÜNTZING, ARNE. Studies on meiosis in diploid and triploid *Solanum tuberosum* L. *Hereditas*, **17**, 223-43. 1932.
- NIELSEN, NIELS. Chromosome numbers in the genus *Hypericum*. *Hereditas*, **5**, 378-82. 1924.
- RISSE, K. Beiträge zur Zytologie der Dipsaceen. *Bot. Arch.* **23**, 266-88. 1928.
- SANSOME, F. W. and PHILP, J. *Recent Advances in Plant Genetics*. Pp. 414. Churchill, London. 1932.
- SIMONET, MARC. *Recherches cytologiques et génétiques chez les Iris*. Diss. Paris, Série A, No. 239, pp. 255-444. 1932.
- TISCHLER, G. Pflanzliche Chromosomen-Zahlen. *Tabulae Biol.* **4** and **7**. 1927 and 1931.

- TISCHLER, G. Über die Verwendung der Chromosomenzahl für phylogenetische Probleme bei den Angiospermen. *Biol. Zentrbl.* **48**, 321-45. 1928.
- WANSCHER, J. H. Studies on the chromosome numbers of the Umbelliferae. I. *Hereditas*, **15**, 179-84. 1931.
- Studies on the chromosome numbers of the Umbelliferae. II. *Botanisk Tidsskrift*, **42**, 49-58. 1932.
- Studies on the chromosome numbers of the Umbelliferae. III. *Botanisk Tidsskrift*, **42**, 384-99. 1933 a.
- Secondary associations in Umbelliferae and Bicornes. *New Phyt.* **33**, 58-65. 1933 b.
- WINGE, Ø. Contributions to the knowledge of the chromosome numbers of plants. *La Cellule*, **35**, 305-24. 1925.
- WOODWORTH, R. H. Cytological studies in Betulaceae. I. *Betula*; II. *Corylus* and *Alnus*. *Botanical Gazette*, **87**, 331 and **88**, 383. 1929.

COMMENTS ON "FLORAL ANATOMY AND ITS MORPHOLOGICAL INTERPRETATION"

By EDITH R. SAUNDERS

Sometime Fellow of Newnham College, Cambridge

(With 50 figures in the text)

IN a recent article which appeared in this *Journal*, A. Arber sets out to discuss various anatomical questions, some of which, perhaps, might be considered to have already been so clearly established as to render such questioning superfluous, as, for example, whether the use of anatomical evidence in phylogenetic morphology can ever be justified(1). One cannot but feel that a statement in the inverse sense, viz. to the effect that without anatomical evidence conceptions regarding phylogeny could never be justified, would have given a more just if less challenging presentation of the position, since the whole of the evidence upon which we have to rely is of an anatomical character, and since the general vascular scheme, no less than the general floral ground-plan, is the outcome of inheritance, and phylogeny is inseparable from heredity. A. Arber is, however, prepared to admit, *with certain qualifications*, that the answer to her query should be in the affirmative. What then are the grounds for this qualified assent? They will be best appreciated if we consider the particular case cited by this writer in which this type of evidence has proved to be reliable, and other examples where, in her view, it is not to be regarded as trustworthy. As coming under the former head she instances the orchid flower, holding, as indeed it would not be possible to deny, that the study of the vascular anatomy in certain members of the Orchidaceae established the fact, previously only a surmise, that the ground-plan of the flower in this family conforms with the fully developed Monocotyledon type. On the other hand she is not prepared to accept that the presence of two bundles in the cotyledon of *Anemarrhena* (Liliaceae) indicates a derivation from two seed leaves. As bearing upon the whole problem, this writer then proceeds to raise the further question whether it is an established fact that the vascular bundles are more "conservative" than the external form of a structure when that structure is in process of

disappearing. She cites as an example which could be classed as an extreme case of such supposed "conservatism" the (in her view hypothetical) solid carpel, and adds, incidentally, the slightly inaccurate comment that the description given by me of the solid carpel *involves* the belief that the carpellary leaf may be reduced to its vascular bundle *alone* (A. Arber's italics)¹. At a later point (*loc. cit.* p. 234) this writer cites the androecium of the Primulaceae as providing another striking example "in which the belief in vascular survival has been responsible for an *artificial and erroneous interpretation*" (my italics).

Let us consider the second of these two examples first. As has long been well established, the sepal midrib bundles in most Primulaceae carry out from the central cylinder conjoined with them a strand which becomes detached before the calyx is exerted and passes up in the corolla (12, p. 266, Figs. 66, 67). Hence the tubular portion of such a primulaceous corolla shows ten *equidistant* bundles, the five on one set of radii providing the petal midribs, and the antepetalous stamen bundles (which in due course become disjoined); those on the alternate radii being the five bundles detached from the sepal midribs whose significance is in question (*loc. cit.* p. 272, Fig. 77). What is A. Arber's comment on these facts? It is to the effect that she has herself observed that these bundles are present in the types which she examined (*Primula vulgaris* and *Anagallis*), that though they are present "*there is no reason to interpret them as stamen bundles*" (A. Arber's italics), that they "appear to be merely laterals belonging to adjacent petals which exist in a state of fusion in the corolla tube but separate with the separation of the corolla lobes." *Ergo*, the reader is led to infer, they *are* laterals. But in what sense is the term "laterals" employed? If it is simply intended to state that these bundles lie between the margins and the midrib of the petals, the statement merely confirms an already well-known fact; if it indicates a *mode of origin* as well as *position*, then from what main bundles are the bundles in question laterally derived? Clearly not from the petal midribs. On the other hand, to suppose that they are not lateral in *origin* as well as in *position* is to imply, on A. Arber's interpretation, that the individual Dicotyledon petal can have more than one vascular system proper to it as an individual floral member, which

¹ Although not inconceivable that a member, carpel or other, which no longer develops as a distinct structure may yet be represented after its disappearance as such, by its main vascular bundle, this conception is not *involved* in my account (see later, p. 161).

is contrary to all experience¹. The only parallel which could be suggested in support of such an explanation is that, as in many families the marginal veins of the *sepals* are carried out from the central cylinder conjoined with the petal midrib bundles, here, in like manner, marginal veins proper to the *petals* might be carried out conjoined with the bundles for an antesepalous stamen whorl now lost. I infer, however, from the trend of A. Arber's argument in the above-mentioned article and elsewhere that she would certainly not entertain this view. We may nevertheless consider what there is to be said for, and against, such an interpretation. In the first place it is to be noted that the general venation scheme of the Dicotyledon calyx differs from that of the corolla in that, whereas distinct marginal veins are exceedingly common in the sepal, similar well-marked marginal veins in the petal are extremely rare. Robert Brown, who first drew attention to this feature in the corolla, cites the Compositae, Goodeniaceae (Goodeniaceae), the rubiaceous genus *Ernodea*, and the two solanaceous genera *Cestrum* and *Datura* as the only instances known to him besides the Primulaceae in which such veins are to be found(2). To this list we can add the Theophrastaceae and some, at least, of the Myrsinaceae. Now in *Cestrum* and *Datura* the petal marginal veins arise by true lateral branching from the petal midribs, and hence do not provide a parallel with the Primulaceae and allied forms. In the Compositae, where we are concerned with a much reduced syngonous flower in which a number of bundles have either already vanished or are in process of disappearing, in the Goodeniaceae and in *Ernodea* in which radial symmetry in the vascular scheme of the more or less syngonous flower is disturbed by an oligomeric ground-plan in the gynoeceum, there is again no strict comparison with the completely isomeric hypogynous flower. Hence, as regards the mode of origin as well as the presence in the corolla tube of twice as many separate and equivalent main bundles as there are petals, the Primulaceae and some allied families appear to stand apart. We have therefore to consider what evidence is available within these families throwing light on this unique vascular ground-plan. *An examination of the genera, Samolus, Soldanella, Steironema (Primulaceae), Deherainia, Clavija, Jacquinia (Theophrastaceae) and some Sapotaceae suffices to show that the current view as to the significance of the five additional bundle systems in the corolla of these*

¹ In the various families I have so far investigated the constant presence of more than one vascular system in a petal is never found in any normal hypogynous flower unless there has been fusion between members.

families rests on evidence which cannot fail to carry conviction to an open mind. Can it be that A. Arber is not cognisant of the facts set out below? The sweeping accusation that the current interpretation of anatomical data of this type is a "game of guesswork" (*loc. cit.* p. 235) should surely be accompanied, if it is to be convincing, by a reasoned statement showing that the evidence on which this interpretation rests will not bear the construction which has been put upon it. *Not only is this evidence not discussed by this writer, it is not even mentioned!* The reader is, presumably, to conclude that there is none. How far this is from being actually the case will be apparent from the facts cited below.

Before entering into details it will be well to emphasise certain important principles underlying floral construction in general. (1) The flower, whatever irregular form its component members may ultimately assume, is fundamentally, with rare exceptions, constructed on a symmetrical ground-plan. (2) The major features in the floral vascular scheme, like the grosser anatomical structures with which they stand in ordered relation, are inherited. In this respect the vascular scheme of the flowering plant may be compared with the circulatory system of the higher animals. The pattern of the ultimate ramifications varies from individual to individual, but the main scheme or ground-plan is fixed and constant. (3) Typically, however the floral scheme may vary in different hypogynous cyclic forms, as many *equidistant* vascular bundles leave the central cylinder for each whorl as there are members in that whorl, *each such bundle becoming the midrib of the member on the corresponding radius*. Midrib bundles of sepals, petals and carpels in many types branch pinnately, those of normal stamens, where a single member is present on a radius, almost always remain unbranched¹. This constant relation between the number of construction radii, of members in the whorls, and of main bundles which become the midribs of the several members provides a useful and reliable means of elucidating the composition of a gamophyllous whorl where outward form gives no certain clue².

With the above generalisations in mind, we are in a position to examine the evidence from the highly instructive genera representing

¹ For exceptions to this condition in modified, and occasionally in normal, members of the androecium see later, pp. 143, 155.

² In making use of this method of analysis, it is of course necessary to bear in mind that as the result of the shortening of the floral axis, temporary fusions are common, both between the main bundles of alternating members and between those of superposed members.

the Primulaceae, Theophrastaceae and Sapotaceae referred to above in which the antesepalous staminal whorl, upon whose existence either in the present time or in the past A. Arber appears to cast doubt, has survived in some measure in outward form. Taken together these types furnish a complete refutation of this writer's argument.

In *Palaquium* (Sapotaceae) this whorl is present and fertile.

In *Samolus* (Primulaceae) (Fig. 1) and in *Reptonia* (Sapotaceae) these members are in the form of antherless filaments. In *Samolus* the antesepalous bundles running up in the corolla tube give off a pair of lateral branches just below the level at which the sterile filaments become detached. The prolonged central (midrib) bundle enters the filament, the laterals diverge to pass into the corolla lobes to right and left respectively, and may branch again. In *Reptonia* the staminode bundles remain unbranched.

In *Deherainia smaragdina* Decne (Theophrastaceae) (Figs. 2, 3) the relations are precisely similar. The antesepalous bundle gives rise to three strands, the central (midrib) bundle entering the short thick finger-shaped staminode which springs from the rim of the corolla tube, and the two laterals usually branching again as they pass up the tube to enter the separate lobes.

In *Clavija macrophylla* Miq. and *Jacquinia armillaris* Jacq. (Theophrastaceae) the main vascular scheme is on precisely the same plan as in *Deherainia*, but in *Clavija* (Fig. 4) the staminodes have assumed the form of large cushions and in conformity with this more expanded shape the vascular system of each staminode has taken on a more definitely foliar character, ending in a brush of short branches. In *Jacquinia* (Fig. 5), in which the staminodes though smaller than the petals resemble them in form and colour, this character is even more pronounced. The grade represented by *Jacquinia* has its precise counterpart among the Sapotaceae in *Labatia*.

In *Steironema* (Primulaceae) (Fig. 12) the process of degeneration in the androecium has progressed further than the stage reached in *Deherainia*. The staminodes are smaller and entirely non-vascular. The antesepalous bundles give off two laterals as in other primulaceous types, but here they end, no central strand being prolonged to supply the short process.

In the great bulk of the Primulaceae, among which are to be included *Primula vulgaris* and *Anagallis*, the types examined by A. Arber, distinct staminodal structures are lacking (compare Figs. 8-11). The bundles detached from the trunk cords on the sepal radii, however, persist, reaching the same grade of development as in

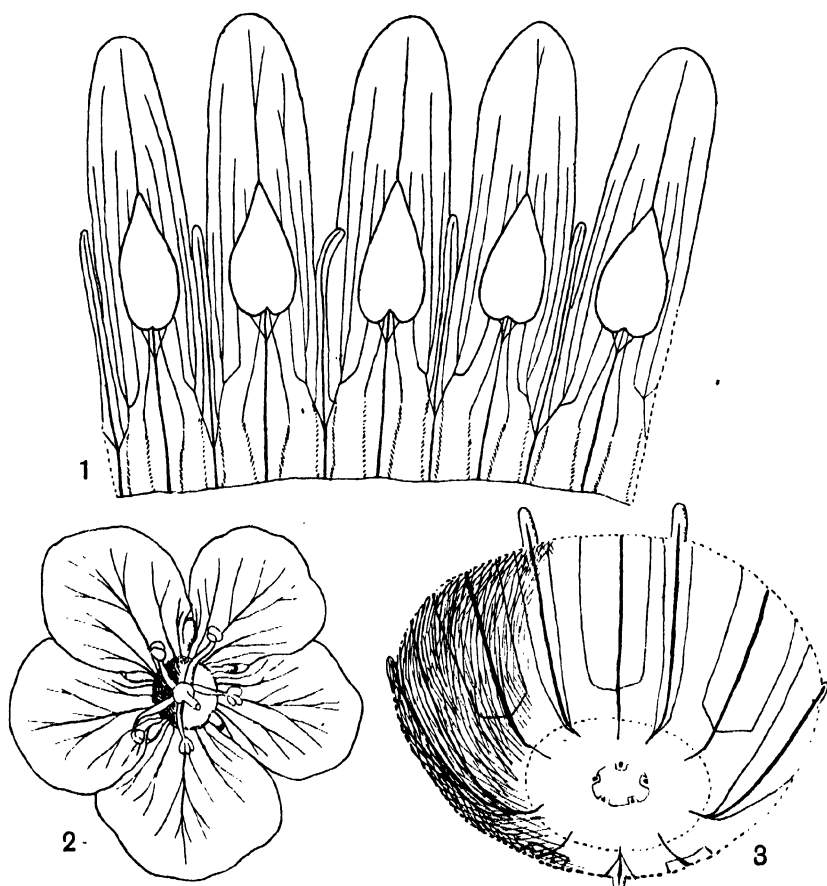


Fig. 1. *Samolus repens* Pers. The corolla split longitudinally and laid flat, viewed from the inner face. The petals showing a median trunk cord giving rise to the petal midrib with its pair of laterals and the bundle for the superposed stamen. Alternating with the petals the antherless staminal filaments into which the alternipetalous bundles, after having given off a pair of lateral branches, are continued. These branches remain in the tissue of the corolla tube, passing above into the petal lobe on either side, and generally, in their turn, giving rise to one or two laterals. Figs. 2, 3. *Deherainia smaragdina* Decne. Fig. 2. The flower viewed from above showing the small staminodes alternating with the petals. Fig. 3. The thick basin-shaped corolla tube which has been cut off below immediately after the emergence from the central cylinder of the ten equidistant bundles for the petals and androecium, and above, immediately below the level of separation of the petal lobes, viewed obliquely from above after being rendered transparent. On the upper rim the five staminodes which alternate with the petals. In the centre the bases of the filaments of the stamens of which the distal portions have been cut away. The vascular scheme as in Fig. 1.

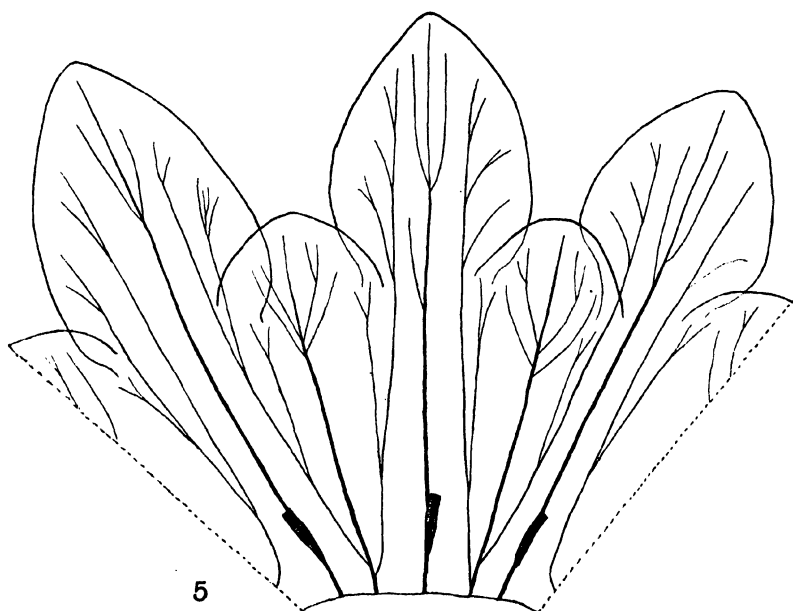
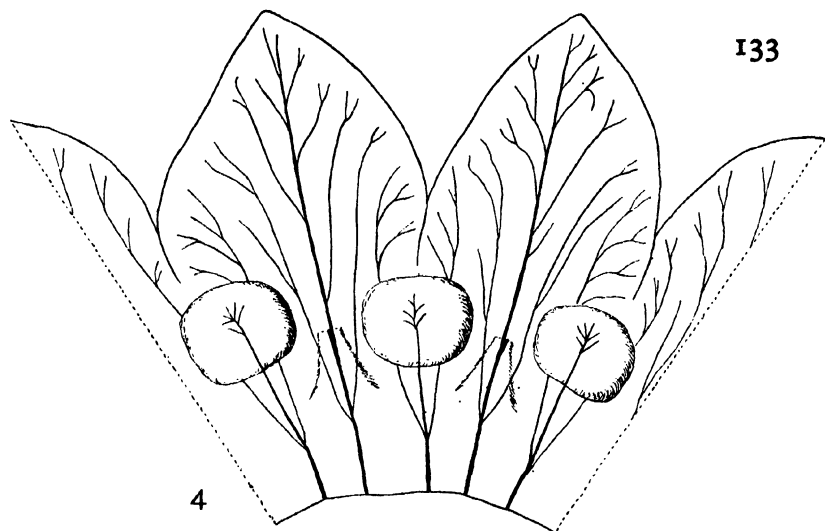


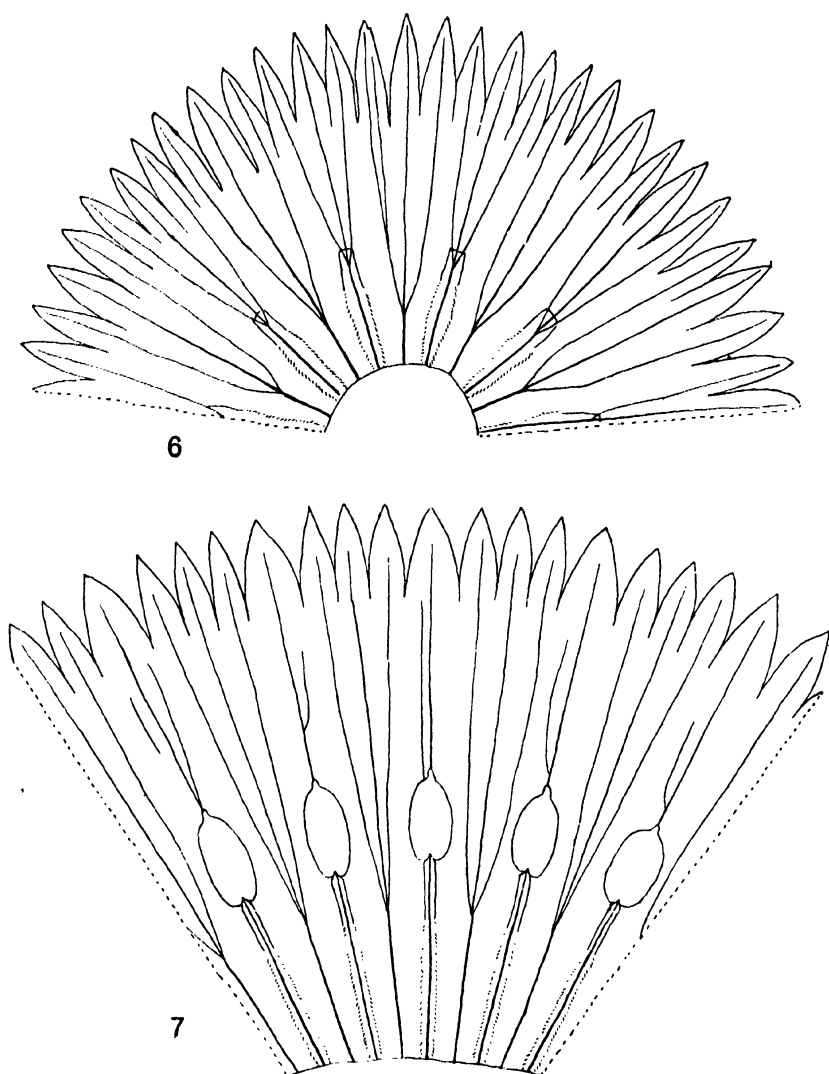
Fig. 4. *Clavija macrophylla* Miq. A portion of the corolla, which has been split longitudinally, viewed from the inner face, showing the cushion-like staminodes alternating with the petals. The filaments of the stamens have been cut away immediately above exertion level in order to expose the whole vascular system of the petals which is derived as in *Deherainia* (see Fig. 3). The mid-vein of the staminodes, in accord with their cushion-shape, terminates in a cluster of short lateral branches. Fig. 5. *Jacquinia armillaris* Jacq. A portion of the corolla treated as in Fig. 4 but the filaments of the stamens have been cut at a higher level. The petal vascular system derived as in Fig. 4 but that of the staminodes, in accord with their petal-like shape, is more fully developed.

Steironema. Laterals are formed but there is no midvein above this level. The same grade is found in the Sapotaceae in *Chrysophyllum*.

In a few forms, among which are *Polyanthus* and *Cortusa*, all five antesepalous bundles are not always developed. When present they behave as in the preceding group, coming to an end at the level at which the two lateral branches diverge.

In *Soldanella* (Figs. 6, 7) the corolla has been described briefly as five-cleft with a fimbriate or laciniate margin. It has been held, like that of other primulaceous genera, to consist simply of five conjoined petals. The more detailed description accompanying the illustration of *S. Clusii* (= *alpina*) in the *Botanical Magazine* for 1820 (vol. 47, Pl. 2163) states that the corolla is "deeply divided into ten laciniae, five of which are three-toothed, and alternate with five simple or undivided ones." On the other hand, Kerner's figures of longitudinally halved flowers of *S. alpina* show in the one half as many as 15 or 16 laciniae⁵. So universally, however, has the above preconception of the composition of the primulaceous corolla come to be taken for granted that the significance of the above facts and their incompatibility with a simple pentamerous ground-plan has been completely overlooked¹. If we examine a number of *Soldanella* flowers in detail, we find in the perfectly symmetrically developed specimen either twenty or thirty laciniae, each furnished with a single median vein. When these veins are traced to their origin it is seen that the vascular system of the corolla tube is on precisely the same plan as that of other Primulaceae. Of the ten equidistant bundles present, five consist of conjoined petal midrib and stamen bundles, the alternate five having been detached from the sepal midribs. When we follow these veins in an upward direction in specimens with thirty laciniae we find that five groups of three laciniae are supplied by the petal midrib and its pair of laterals, and that the five alternate triplets receive an antesepalous bundle and its pair of laterals (Fig. 6). In the only specimen I was able to examine having twenty laciniae—a flower of *S. pusilla*—I found the laterals of the petal midribs so short and feebly developed that no formation of corresponding laciniae (or no corresponding segmentations, according to the way in which one views the developmental process) had taken place (Fig. 7). Each petal had remained entire. On the other hand, each of the five antesepalous bundles had branched in the usual way, each group of the three resulting strands supplying one of the groups of three laciniae. If the above description of *S. alpina* in the *Botanical Magazine* may be taken to be correct as regards the radial disposi-

¹ See Postscriptum, p. 170.



Figs. 6, 7. Corollas of species of *Soldanella* treated as in Fig. 1. Fig. 6. *S. alpina* L. A corolla of thirty laciniae consisting of ten alternate triplets, one set of five supplied by the petal midribs and their laterals, the other set by the whorl of antesepalous bundles and their laterals. Fig. 7. *S. pusilla* Baumg. A corolla of twenty laciniae consisting of five slightly larger entire segments alternating with five triplets of slightly narrower ones, the entire segments (= petals) supplied by the petal midribs which form very weak lateral branches, the triplets, like the corresponding set in *S. alpina*, by the whorl of antesepalous bundles and their laterals. [For the sake of clearness the anthers have been cut away in Fig. 6.]

tions of the triplets and the single laciniae, respectively, in the specimen figured¹, then it appears that a corolla with twenty laciniae can occur in which the segmentation scheme is the converse of that observed in the above flower of *S. pusilla*, the petal midribs branching sufficiently vigorously for the petals to become trifid, while the antesealous bundles must evidently branch so feebly that the corresponding sectors remain entire. Laciniae numbers ranging between twenty and thirty result when development is more vigorous on some radii than on others; numbers higher than thirty occur when the lateral veins in their turn branch and there is corresponding further secondary segmentation. These variations appear to be independent of heredity, the numbers varying from individual to individual (and probably from flower to flower) in each of the three species examined (*alpina*, *montana* and *pusilla*), but the presence of ten equidistant and equivalent vascular systems is constant, as in the other genera in the family.

The above facts show that the *Soldanella* corolla is a composite structure composed of two floral whorls moulded into one consisting of the petals and the outer stamen whorl converted into similar petaloid structures, the two whorls being united in the same manner as the two stamen whorls in a flower with a monadelphous androecium. The resulting compound structure is diagrammatic in its simplicity; its interpretation is equally plain and inescapable.

The structural features in the above-mentioned genera, which can be observed with the utmost ease, reveal certain phases of the evolutionary history of the Primulaceae and allied forms in chapters so plainly writ that we can follow the whole story. Yet this is one of two cases picked out by A. Arber as justifying her attitude of wholesale condemnation of current interpretations of floral evolution based on anatomical details as a "game of guesswork."

Briefly our story runs as follows:

In some ancestral stock to which the Primulales trace back, outward form and the vascular ground-plan showed the presence of two staminal whorls, the bundles for the outer whorl passing out from the central cylinder conjoined with the sepal midrib bundles, those for the inner whorl being similarly conjoined with the petal midrib bundles. At some point the antesealous whorl began to

¹ Caution in accepting this description until it is confirmed by the examination of a number of specimens seems advisable in view of the fact that it would be written with a preconceived idea in the mind, and that observation shows that the *depth* of segmentation is not invariably greater between the different sectors than between the laciniae of one sector.

degenerate. No anthers were formed. The sterile filaments became smaller. Having lost their capacity to function reproductively, the members of this whorl assumed, or reverted to, a more typically foliar condition in that the corresponding bundles, originally (as typically in members of the androecium) unbranched, now showed pinnate branching, the midvein alone entering the sterile staminal member as it became exerted, the lateral branches continuing their upward course into the free lobes of the corolla (*Samolus*, *Clavija*, *Jacquinia* and *Deherainia* types).

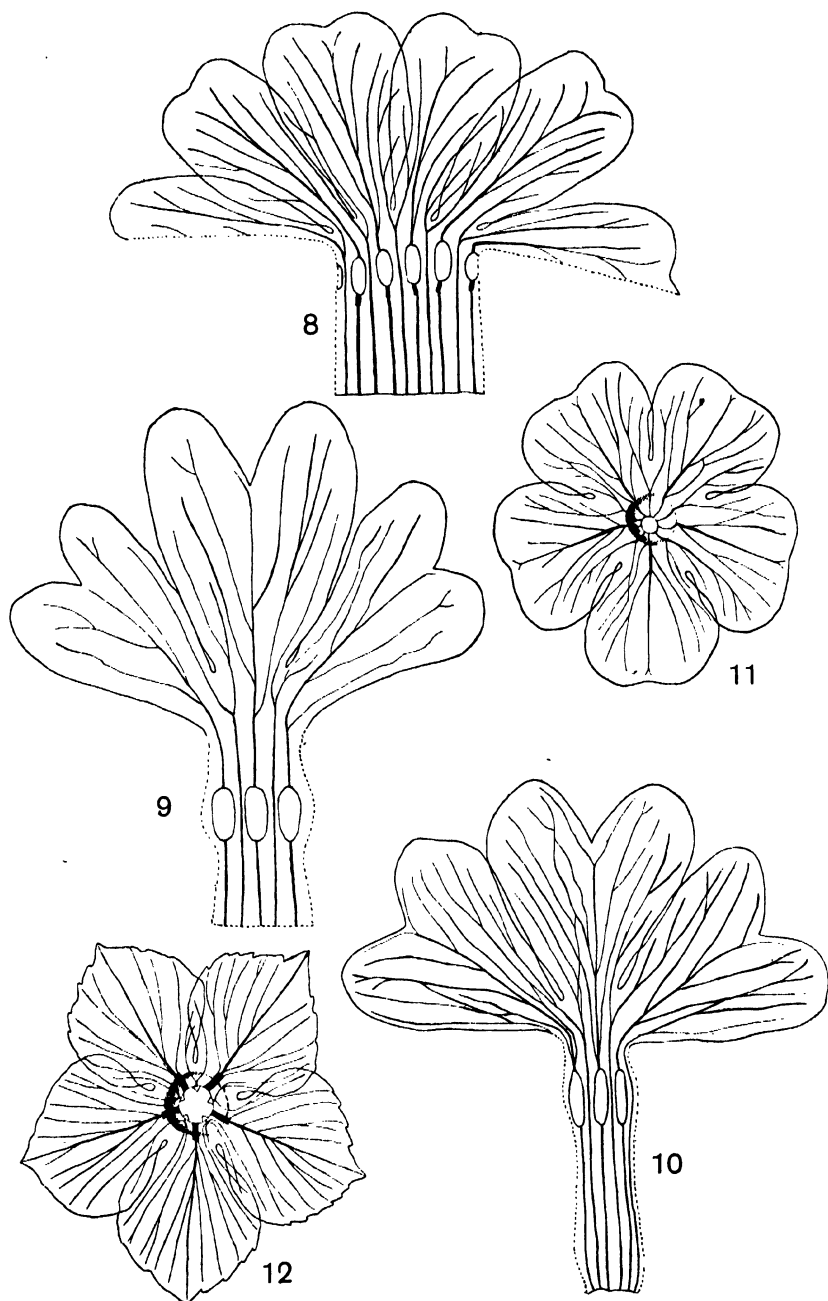
In certain genera these staminodes became further reduced in size, and destitute of vascular tissue, the antesealous bundles giving rise as before to a pair of laterals but ceasing to be prolonged into the central strand which in earlier stages of degeneration passes into the staminode (*Steironema* type).

A further stage in the downward process along this same line of descent, or, it may be, divergence from this line at some earlier point, has produced the type seen in most primulaceous and some (? all) myrsinaceous forms, in which the antesealous stamens are no longer developed as separate structures of characteristic form. Nevertheless the corresponding bundles persist, but they are no longer prolonged above the level of origin of the lateral branches (dominant type of to-day).

Directly descended from this last grade are those few forms in which these antesealous bundles have begun to disappear, one or more being found to be lacking in particular flowers, individuals or species (*Cortusa*, *Polyanthus*).

Divergence in yet another direction led to the *Jacquinia* and *Soldanella* types, the most interesting of all from the present point of view. Along this line of descent, after the antesealous staminal members had ceased to function as stamens and had developed a characteristically foliar vascular system (midrib and laterals), they, together with these systems, not only persisted entire, but each became incorporated into the corolla as a distinct sector with a recognisable outward form.

Outlined above we have then the proof which A. Arber so unhesitatingly declares not to exist, that in the Primulales and some Sapotaceae an antesealous stamen whorl has been "lost" while the corresponding bundles still remain. It is clear that in these families the vascular ground-plan of perianth and androecium does not depart from the fundamental scheme of the isomerous pentamerous six-whorled Dicotyledon; that modifications of the outer



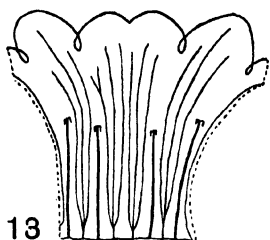
Figs. 8-12.

staminal whorl have taken place and have followed a different course in different lines of descent, leading along some lines to disappearance of the members of this whorl as separate morphological entities, and along others to the assumption of petal-like form, colour and venation pattern. To accept the somewhat meagre evidence available in the Orchidaceae and to reject a much more complete body of facts of a comparable nature in the Primulaceae and allied forms is on the face of it wholly illogical. Indeed it is difficult to avoid the conclusion that A. Arber is content either not to seek, or to ignore, facts which are fatal to her would-be destructive criticisms. For the further one extends one's observations the more evident it becomes that persistence of the vascular bundles of suppressed members of the androecium is by no means of rare occurrence. Furthermore, one finds in many gamopetalous types that degeneration involving loss of functional activity in *individual* stamen members may be accompanied, precisely as when the *whole whorl* is suppressed, by the formation of lateral branch systems derived from the main bundles proper to the stamen members, these systems running in the corolla as in the Primulaceae, Theophrastaceae and some Myrsinaceae (e.g. *Ardisia*). Especially is this latter feature to be looked for in types in which the bundles destined for the androecium normally run for some distance up the corolla tube before the exertion level is reached. This condition is realised in various genera belonging to Labiatae, Verbenaceae, Gesneriaceae, Acanthaceae and Scrophulariaceae, which, as will appear from what follows, furnish a further refutation of A. Arber's whole argument.

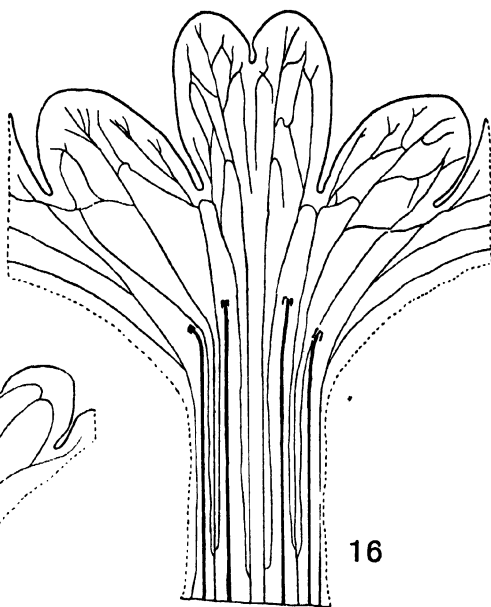
LABIATAE (Figs. 13-26 and 28-32)

As is well known, the bulk of the genera in the Labiatae have one whorl of stamens composed of two antesepalous pairs (Figs. 13-17), but in *Rosmarinus* and *Salvia* only the antero-lateral pair develop.

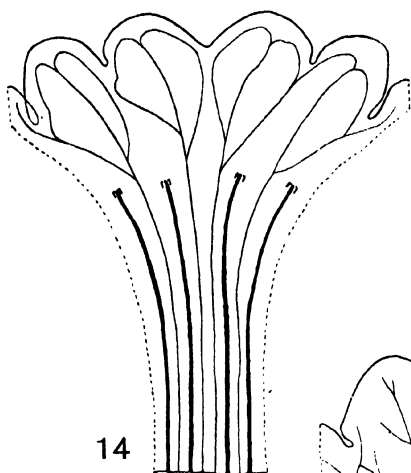
Fig. 8. *Hottonia palustris* L. The corolla split longitudinally and laid flat, viewed from the inner face. Fig. 9. *Primula frondosa* Janka. Three petals from a corolla treated as in Fig. 1. [For convenience of arrangement the basal portion of the tube has been cut away.] Fig. 10. *P. farinosa* L. Three petals from a corolla treated as in Fig. 1. Fig. 11. *Androsace coronopifolia* Andr. The corolla viewed from above. [For simplicity the stamens are not represented.] In *Primula frondosa* the petal midrib systems and the intervening antesepalous systems remain distinct. In the other three types they anastomose. Fig. 12. *Steironema ciliatum* Rafin. The corolla viewed from above. Projecting into the central space the five small non-vascular staminodes. On the alternate radii the cut-off filaments of the stamens. The branch system derived from the midrib and the marginal vascular systems of the petals remain distinct as in *Primula frondosa*.



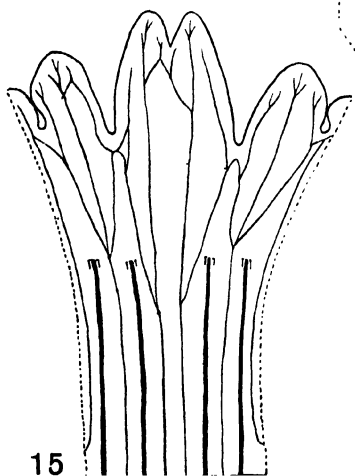
13



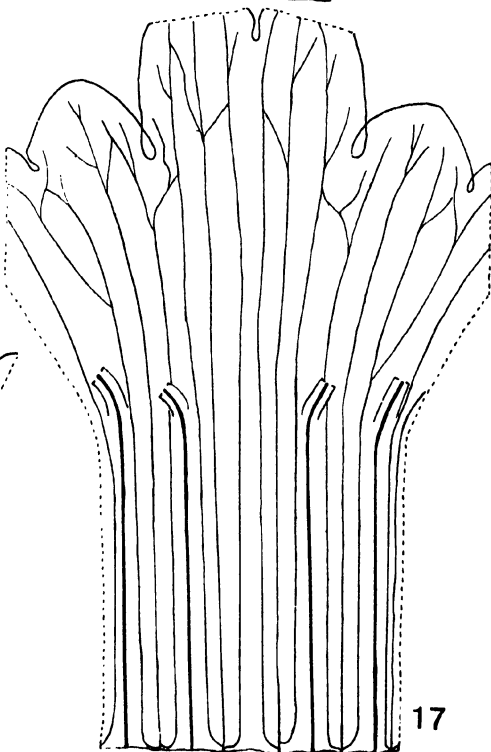
16



14



15



17

Figs. 13-17.

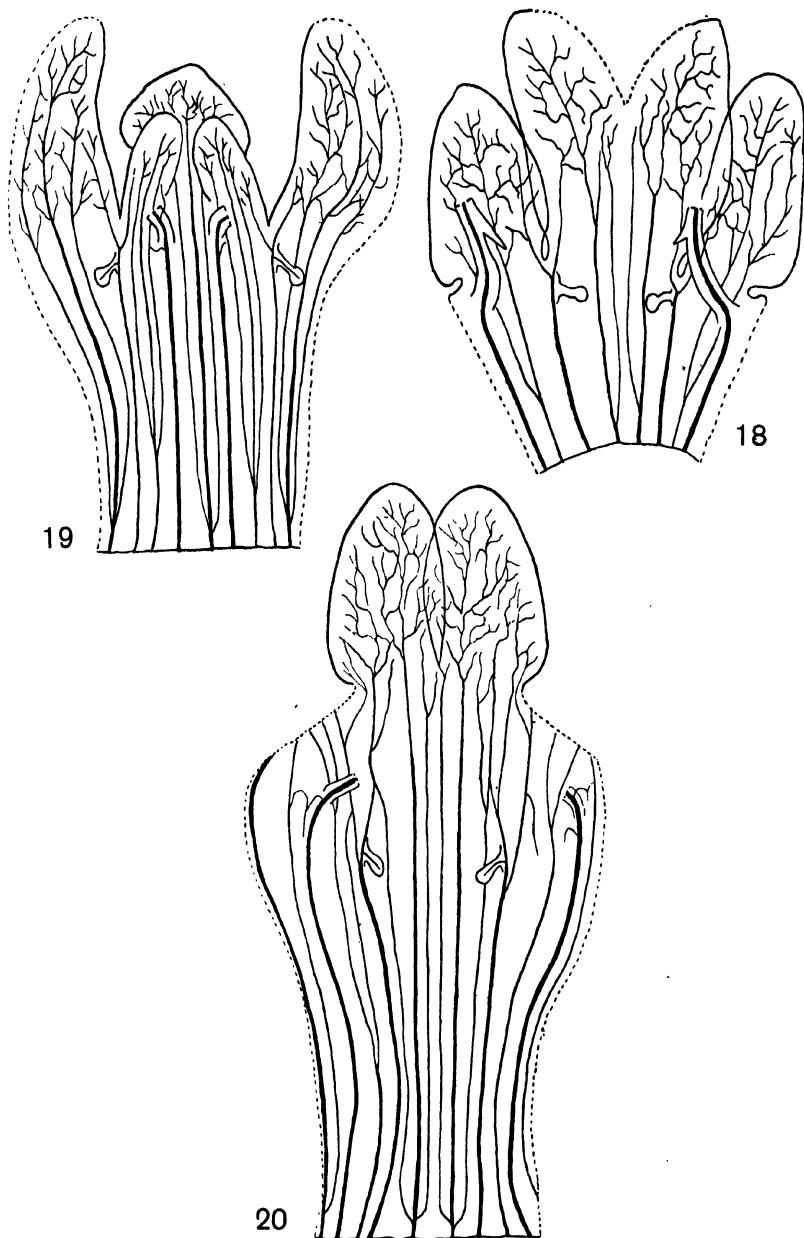
In both these genera the "lost" postero-lateral pair are represented by microscopic structures terminating in a rounded or lobed enlargement (Figs. 18-24). In neither two-stamened nor four-stamened types is there any outward trace in normal flowers of the fifth (posterior) member of the whorl.

Sections of the flowers of different four-stamened genera show that the vascular bundles for the four members of the androecium turn out from the central cylinder independently and later than those for the petals with which they alternate (Figs. 28, 29). No antesealous bundle emerges in the midline at the back. It is hardly to be doubted that the complete fusion of the two postero-lateral petals into a hood-shaped structure has led to the suppression of this bundle as well as of the member which it served.

Examination of sections of the flowers of such species of the two-stamened genera as chanced to be available at the end of the season¹, viz. *Salvia azurea* Lam., *S. Chamaedrys* Willd., *S. glutinosa* L., *S. Horminum* L., *S. leucantha* Cav., *S. patens* Cav., *S. rutilans* Carr., *S. schiedeana* Stapf, *S. Sclarea* L., *S. splendens* Ker-Gawl, *S. virgata* Ait., and *Rosmarinus officinalis* L., showed that normally four bundles turn out from the central cylinder in line with the antero- and postero-lateral pairs of sepals to serve the androecium precisely as in those genera in the family which still develop four functional stamens (Figs. 30, 31). At this level the vascular scheme of two-stamened and four-stamened types is alike. In both classes the four bundles continue upwards in the corolla tube. In the four-stamened genera they pass unbranched into the corresponding filaments (Figs.

¹ It may be noted in passing that not only the flowers of Labiatae but those also of Verbenaceae, Gesneriaceae and Acanthaceae upon which the present observations were carried out were taken at random from among those still available in October and November.

Figs. 13-17. The corollas of five labiate genera split down the front and laid flat, showing variations in branching of the petal midribs in the tube region, and the unbranched bundles of the stamen members. The lateral veins of the anterior petal are seen, when present, near the cut edge on each side, but the midrib which lies in the plane of the split is omitted except in Fig. 17. Fig. 13. *Micromeria Juliana* Benth. All five petals, though small, have both lateral veins. Fig. 14. *Elsholtzia Stauntoni* Benth. All five petals are without lateral veins in the region of the tube. Fig. 15. *Prunella laciniata* L. var. *alba*. The postero-lateral petals with only the outer laterals which arise high up in the tube. Those of the anterior and of the antero-lateral petals arise near the base of the tube and above the exsertion level of the stamens, respectively. Fig. 16. *Hyssopus officinalis* L. The corolla vascular scheme as in Fig. 15 but the laterals arise at different levels. Fig. 17. *Lavandula spica* L. The vascular scheme as in Fig. 13. [Magnified equally.]



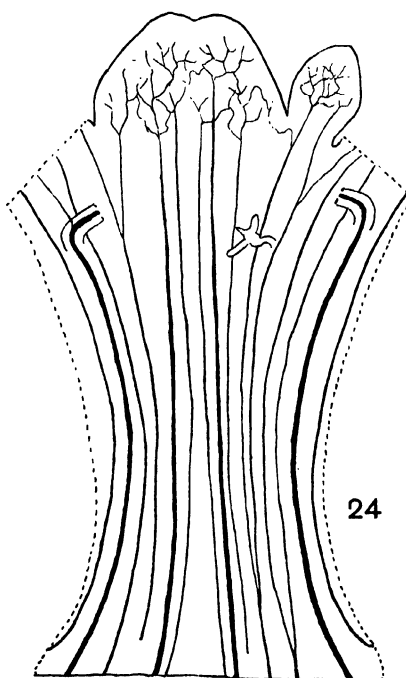
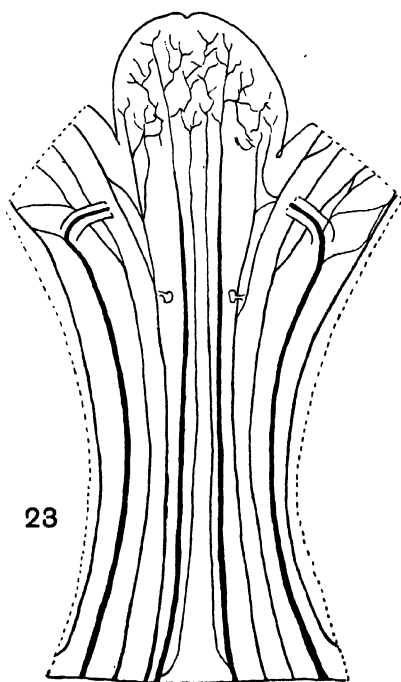
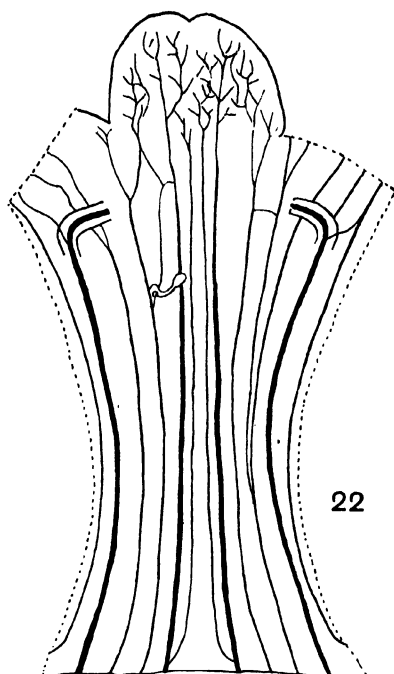
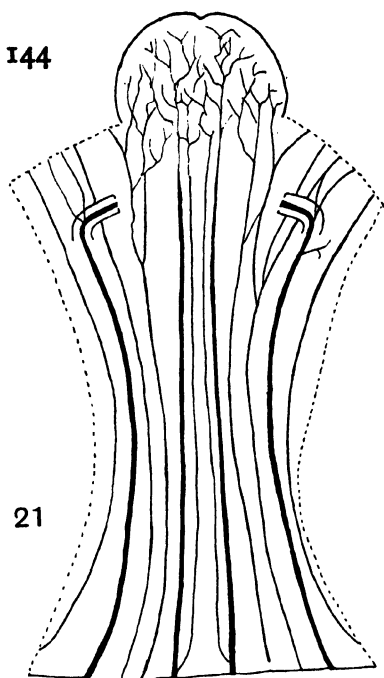
Figs. 18-20.

13-17). In all the above-mentioned species of *Salvia* except *glutinosa*, in which the filaments are particularly stout, the bundles for the two functional stamens give rise regularly or occasionally to one or more lateral strands which continue upwards as the main bundle turns at right angles at the top of the corolla tube just before the filaments are exerted (Figs. 19-24). Other branches are also occasionally given off at a lower level. These branches usually anastomose with strands derived from the nearby chief lateral of the petal on either side. In *Salvia* the stamen bundle again branches after reaching the top of the filament, one of the two strands formed turning along the arm of the connective bearing the half anther, the other along the sterile arm in which it may again branch further as is well seen in *S. splendens* (Fig. 26). Here we have another instance where a distinctly foliar type of venation is developed when a fertile structure, having typically an unbranched vascular system, is modified into a sterile structure. This potentiality is not uncommon in members of the androecium which still function normally, as is evidenced by the number of species in which some degree of petaloidy of the connective has been observed.

In both *Rosmarinus* and *Salvia* the bundles proper to the two, here almost vanished, postero-lateral staminal members also give rise to a branch system at, or near, the exertion level. One of these branches sooner or later bifurcates preparatory to the separation of the corolla lobes, the two resulting strands passing respectively into the lobe on either side where they anastomose with the system proper

Fig. 18. *Rosmarinus officinalis* L. A corolla, from which the anterior petal and the distal portion of the stamen filaments have been cut away, split completely down the front and partly down the back. The bundles for the two postero-lateral petals have developed inner laterals only, those for the antero-lateral petals, outer laterals only. Staminodes without a differentiated vascular strand. Fig. 19. *Salvia Horminum* L. A corolla split longitudinally down the back. In the centre the anterior petal flanked by the antero-lateral petals which, in turn, are flanked by the larger postero-lateral petals forming the helmet-shaped upper lip. The midribs of all five petals give rise to a pair of lateral branches in the region of the tube. The bundles of the two stamen filaments which have been cut away just above exertion level show a fine lateral strand just below this level. Staminodes with vascular strand representing the termination of the corresponding main bundle. Above this level the pair of laterals which diverge into the neighbouring petal lobes but which at first are conjoined. Fig. 20. *S. Chamaedrys* Willd. A corolla, from which the anterior and antero-lateral lobes and the exerted stamens have been cut away, split down the front. The bundle system of the anterior and two postero-lateral petals as in Fig. 19, that of the two antero-lateral petals less well developed. Both stamen and staminode main bundles branch in much the same manner as in Fig. 19.

I44



Figs. 21-24

to the petal (Figs. 18-24). In *Salvia* species in which the staminode is somewhat above the minimum size the corresponding main bundle is usually prolonged beyond the branching point as a midvein which, turning more or less at a right angle as in the functional stamens, enters the little structure as it becomes exserted (Figs. 19, 20). In some species this vein is differentiated up to the apex of the staminode; in others the basal portion connecting with the main trunk remains unligified; in others, again, it may remain undifferentiated throughout. In species in which, on the other hand, the staminodes are extremely minute this midvein is no longer traceable (*S. rutilans*, *S. Sclarea*, *S. schiedeana*, Figs. 21-24). It had also disappeared in all the flowers examined of *Rosmarinus* (Fig. 18), although the staminodes here are larger than in most of the species of *Salvia* examined. Two of the above types, *S. schiedeana* and *S. rutilans*, have an additional special interest in the present connection, the degeneration process in the androecium having progressed further in individual flowers than the point at which the bulk of *Salvia* species have become stable to-day. Of the last seventeen flowers of the season obtained from *S. schiedeana* three were found to possess both staminodes, four lacked both, and in the remaining ten one of the two was present. *In those in which one or both staminodes were lacking the corresponding main bundles were present in every specimen investigated* (Figs. 21-24). That these bundles will in their turn gradually

Figs. 21-24. *Salvia schiedeana* Stapf. The corolla tube split longitudinally down the front after the lobes of the anterior and antero-lateral petals and the filaments of the stamens have been cut away. [The midrib bundle of the anterior petal which lay along the line of the split is not represented.] In the centre the midrib bundles of the two postero-lateral petals with the inner laterals only. In succession outwards, on each side, the bundle proper to the postero-lateral staminode, the midrib of the antero-lateral petal, the bundle of the antero-lateral stamen from which lateral branches are given off near where it curves just before the filament becomes exserted, and at the cut edge a lateral vein of the anterior petal. The staminode bundles give rise to branch systems which anastomose with those of the petal on either side, but are not prolonged into the exserted structure. Fig. 21. A corolla in which both staminodes are lacking. Fig. 22. A corolla with one staminode. Fig. 23. A corolla with two staminodes. Fig. 24. A slightly abnormal corolla. The left staminode has disappeared. The corresponding member on the right is represented by an unusually large exserted structure seated on one branch of the staminode bundle and a petaloid expansion in which the other branch ramifies. The right postero-lateral petal midrib has an outer as well as an inner lateral; the basal portion of this lateral and also that of the left staminode bundle are no longer traceable. The inner laterals of both postero-lateral petal midribs arose below the level of exsertion of the corolla and hence appear disconnected.

disappear is evident from the fact that some were observed to have begun already to decline in development. This weakness begins at the level of origin and extends upwards for varying distances. In the earliest phase the bundle no longer emerges independently from the

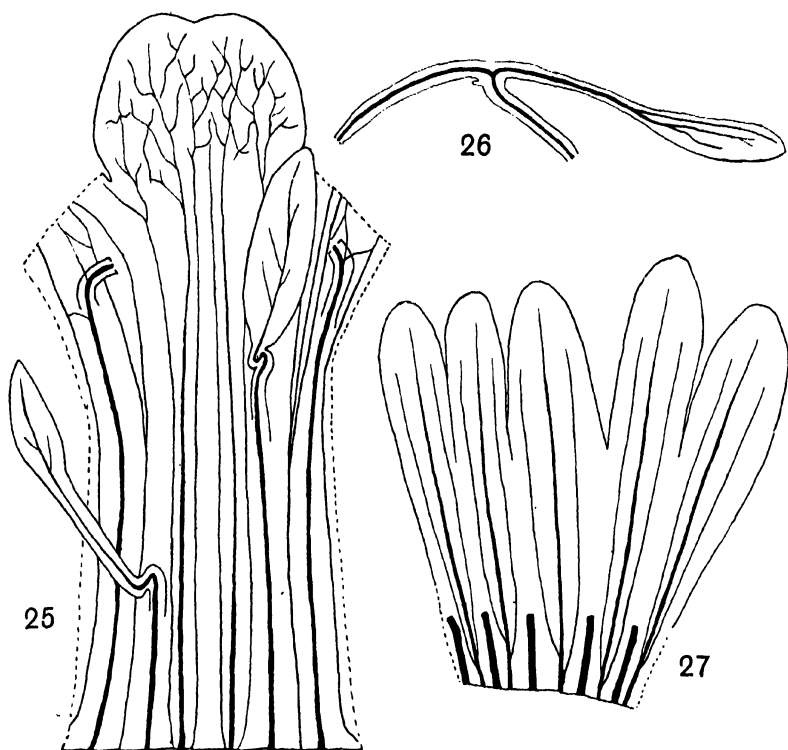
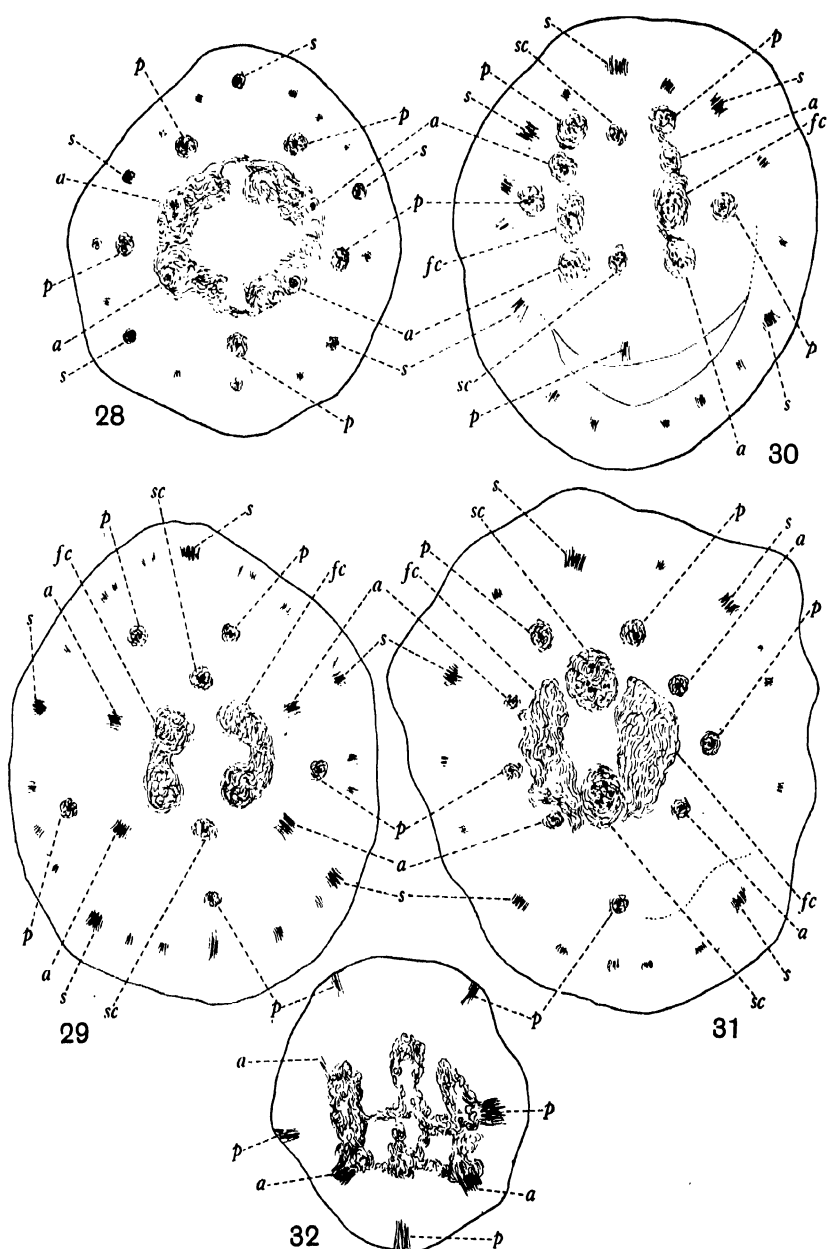


Fig. 25. *Salvia schiedeana* (continued). A corolla from a flower in which both staminodes have become leaf-like in form, treated as in Figs. 21-24. Increase in width of the staminode is accompanied by pinnate branching of the bundle. Fig. 26. *S. splendens* Ker-Gawl. A stamen showing pinnate branching of the bundle in the sterile arm of the connective which forms a wide, flattened plate. In the arm (on the left) from which the anther has been removed the bundle is unbranched. Fig. 27. *Geunsia farinosa* Blume. The corolla, which is nearly strictly actinomorphic, with the basal portion of the filaments of the five stamens which are all fertile. [Drawn from herbarium material.]

central cylinder but is conjoined with the emerging midrib bundle of the neighbouring postero-lateral petal, only becoming dissociated after exsertion of the corolla tube in which it then follows a normal course. In a more advanced stage the part of the strand immediately above the level of separation, i.e. the lowest independent portion, is

no longer traceable, differentiation only beginning at some indeterminate point higher up the tube, the bundle thereafter continuing its upward course and branching in the usual way (Figs. 21, 24). Among many flowers examined of *S. rutilans* one was found with only a single staminode. *In this exceptional flower the vascular scheme was identical with that of those having two staminodes.* Here we have again undeniable proof that vascular bundles can, and do, persist when all trace of the outward form of the corresponding member has disappeared. In the staminodeless flowers of *S. schiedeana* (Fig. 21) we see exhibited the same vascular relations, allowing for the differences in the floral formula, as in the bulk of the Primulaceae and in some Myrsinaceae, while the few genera possessing staminodes in the former family and in Theophrastaceae have their counterpart in the bulk of *Salvia* species and in *Rosmarinus*.

It is natural that the *level* at which branching occurs in these androecium systems should bear some relation to the form of the corolla. In Primulaceae and Theophrastaceae where the tube is short it necessarily occurs at a low level; in the long-tubed labiate corolla it usually takes place near the top of the tube. In both types separation of the corolla lobes involves divergence of the resulting strands in their further course, one or more passing into the adjacent lobe to right and left. It scarcely needs to be added that, as in the Primulaceae and Theophrastaceae, no ground can be advanced for looking upon the persistent androecial vascular systems in *Salvia* and *Rosmarinus* as forming a phylogenetically integral part of the system proper to the neighbouring petals. In the first place, as stated above, the bundles emerge from the central cylinder on the radii proper to the stamens and consequently in line with the sepals, a sufficient indication of their true nature. Furthermore, the vascular system proper to each of the five petals in both four-stamened and two-stamened genera conforms in its main features to one uniform scheme. This in its fullest expression consists of a midrib bundle turning out on the petal radius and one chief pair of laterals which may arise either at the base of the corolla tube or at some distance up it. The anterior petal almost invariably forms both laterals, but in each pair of the other petals one of the two laterals, the corresponding one in the two petals of a pair, but the one nearer the front in some species, that nearer the back in others, may fail to develop. Thus in species of *Lavandula* (*L. spica* L. (Fig. 17), *L. dentata* L., *L. abrotanoides* Lam.) and in *Micromeria Juliana* Benth. (Fig. 13) all five petals develop both laterals at the base of the tube. In the



Figs. 28-32.

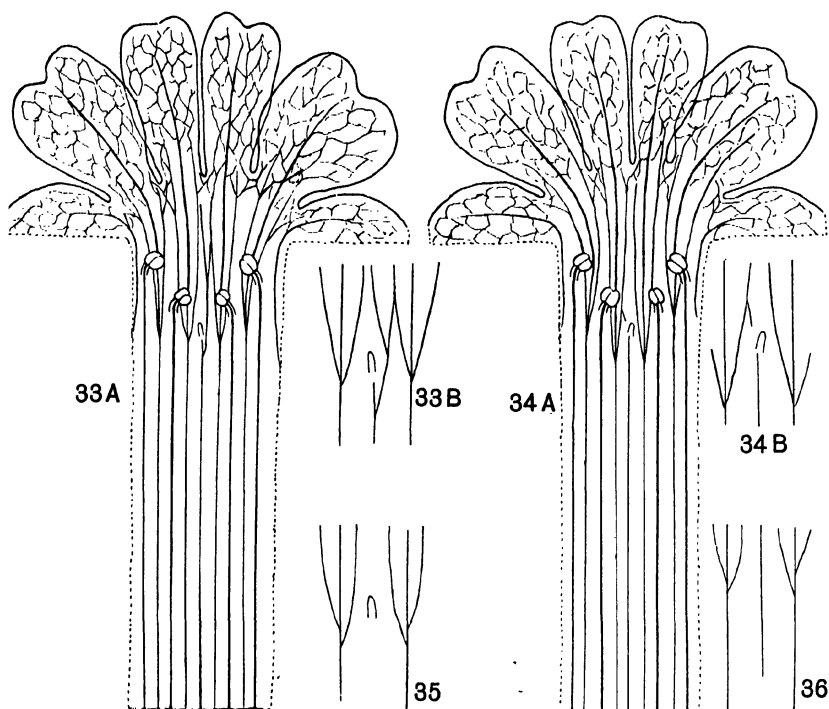
narrow tube of *Elsholtzia Stauntoni* Benth. (Fig. 14) no laterals were formed. In the two postero-lateral petals of *Hyssopus officinalis* L. (Fig. 16) and *Lamium album* L. the laterals nearer the front only were present. In *Salvia leucantha* and *S. schiedeana* the reverse condition was found to be general (Figs. 21-23). But these incomplete expressions of the general scheme occur alike in two-stamened and four-stamened types. There can, in short, be no possible ground for suggesting, by any turn of phrase, that in the corollas of *S. schiedeana* two of the anteseptalous bundles are mere petal laterals while the other two are genuine stamen bundles.

VERBENACEAE (Figs. 27 and 33-36)

In the Verbenaceae in which zygomorphism is much less pronounced than in the Labiatae, being, indeed, in some genera hardly appreciable, the degeneration of the androecium is correspondingly less marked. In a few types, as in *Geunisia farinosa* Blume, the full complement of five stamens is still formed, all five being functional (Fig. 27). The first step in the process of degeneration is exemplified in *Citharexylum quadrangulare* Jacq., in which the posterior staminal member may bear a small-sized functional anther or may be reduced merely to a filament, in which the bundle proper to the member terminates. Showing still further reduction is a large group of forms in which the posterior staminode has been completely lost together with the corresponding bundle, e.g. *Caryopteris Mastacantha* Schau, *Clerodendron fallax* Lindl., *C. Fargesii* Dode, *C. foetidum* Bunge, *C. splendens* G. Don, *Lantana delicatissima* Poit., *Lippia nodiflora* Michx., *Vitex incisa* Lam., many species of *Verbena*. Other *Verbena*

Figs. 28-32. All from transverse sections of flowers of Labiatae. Figs. 28, 29. *Lamium album* L. Fig. 28. The perianth bundles have turned out from the central cylinder; those for the four stamens, though already defined, have not yet emerged. Fig. 29. The stamen bundles have turned outwards. The midrib bundles for the two sterile carpels are now defined but those for the two fertile members are not yet differentiated. Fig. 30. *Rosmarinus officinalis* L. A slightly older stage than that seen in Fig. 28. Fig. 31. *Salvia glutinosa* L. From a flower with both staminodes. An intermediate stage between those shown in Figs. 29 and 30. Fig. 32. *Salvia schiedeana* Stapf. From a staminodeless flower after exsertion of the calyx. In addition to the bundles for the five petals and the two antero-lateral stamens one staminode bundle is seen about to turn out from the central cylinder (above, on the left). The other staminode bundle was also present in the tube region of the corolla, but the basal portion either having failed to become differentiated or being fused with the neighbouring postero-lateral petal bundle does not appear at this level. *s*, sepal midrib bundle; *p*, petal midrib bundle; *a*, stamen bundle; *fc*, fertile carpel bundle; *sc*, sterile carpel bundle.

species have not yet reached this stable end-stage but produce some flowers showing some trace of the posterior stamen member. In



Figs. 33-36. *Verbena canadensis* Britton. Fig. 33 A. A corolla split longitudinally down the front. In the centre line the bundle of the posterior member of the androecium which is reduced to a small tongue-shaped structure. The exerted structure is without vascular tissue, the main bundle terminating just below the exertion point. A branch from this bundle is continued to a higher level and branching again anastomoses with the nearby lateral of the neighbouring petal. Fig. 33 B. The staminode of the preceding figure and the vascular system of the surrounding area more highly magnified. Fig. 34 A. The corolla of another flower treated as in Fig. 33 A. The staminode bundle ceases just below exertion level as in Fig. 33 A. Above, on the left, extending downwards from the nearby petal lateral is a short strand ending freely which is probably the surviving distal portion of an anastomosing branch of which the basal portion is undifferentiated or missing. Fig. 34 B. The staminode of the preceding figure and the vascular system of the surrounding area more highly magnified. Fig. 35. The same from a flower in which the staminode remnant has persisted but the corresponding vascular bundle is no longer traceable. Fig. 36. The same from a flower of another race in which the staminode remnant has entirely disappeared but the corresponding bundle is still differentiated in the upper part of its course.

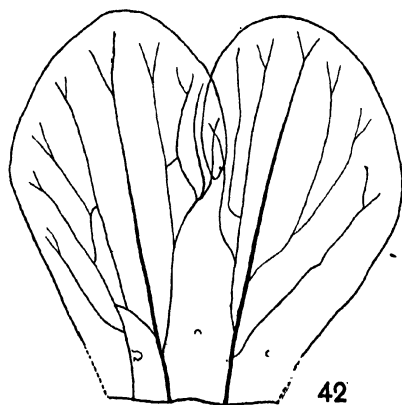
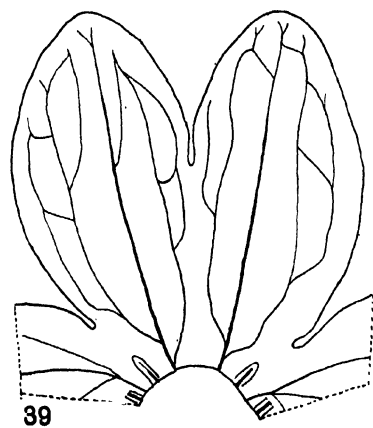
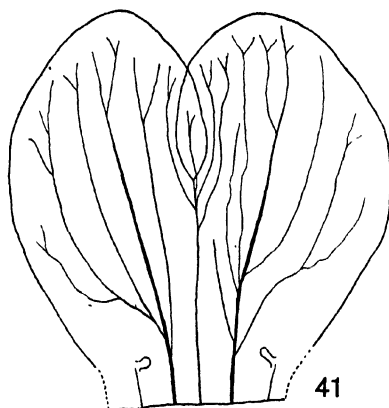
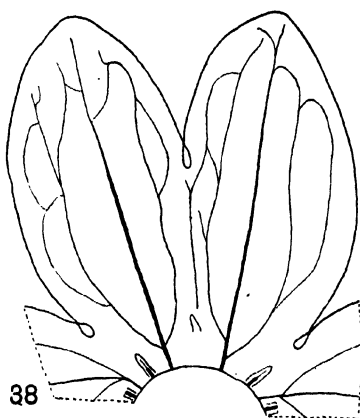
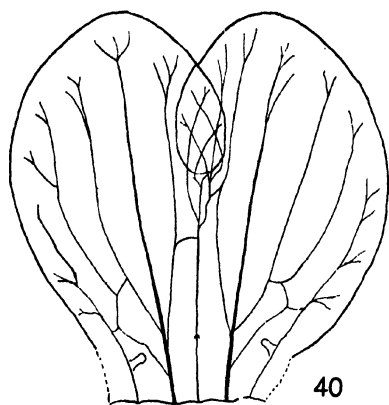
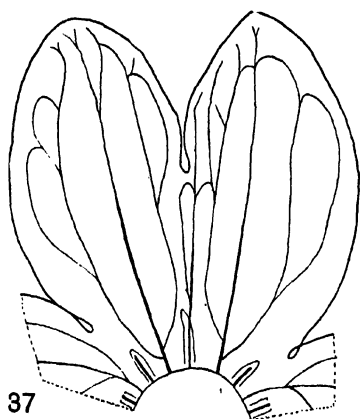
these higher grade flowers in *V. canadensis* Britton the posterior stamen was represented by a minute non-vascular tongue-shaped

outgrowth or by a mere "pimple." The corresponding bundle was often no longer to be traced (Fig. 35) but in some specimens it was developed normally up to a point just short of the level of exertion. Here the midvein always ceased, no strand passing into the staminode. But in some flowers a lateral branch was given off which continued to a higher level and anastomosed with a nearby petal lateral (Figs. 33, 34). Here we see, in perhaps its simplest form, the development of a branch system which, as has been shown, is so frequently associated with degeneration from the fertile to the sterile condition. In another similarly unstable race probably to be referred to the same species¹ the higher grade flowers similarly showed persistence of the bundle after the corresponding member had been wholly "lost," the posterior stamen bundle being traceable for some distance in the upper region of the tube though no corresponding structure, no indication even of the exertion point, was discernible. The degeneration process had, however, evidently begun to involve the vascular system for in this case the bundle made its first appearance some way up the tube, the basal portion connecting with the central cylinder being no longer traceable (Fig. 36). The stage reached in such specimens is thus similar to that exhibited by the bundles for the staminodes in some flowers of *Salvia schiedeana* (see above p. 144, Figs. 21, 24).

GESNERIACEAE (Figs. 37-42)

Modification of some members of the androecium into staminodes and "loss" later of some of these modified members by suppression are characteristic features of many genera of the Gesneriaceae. Two types in which stages in the process of degeneration can be followed again afford evidence of the persistence of the bundles of members which have become suppressed and of the development of branch systems by these bundles following upon loss of the reproductive function. In *Petrocosmea nervosa* Craib (Figs. 37-39) only the two antero-lateral members of the androecium, which are exerted at the base of the short corolla tube, are functional. The two postero-lateral members, which are reduced to small tongue-shaped outgrowths, appear always to be present together with the corresponding bundles which in some flowers are differentiated throughout (Figs. 37, 39), in others may remain unligified for some (Figs. 38, 39) or all of their length. The posterior staminode may attain to a somewhat larger size than the postero-lateral pair. Or it may be observed

¹ Though passing under the name *V. Drummondii-montana*.



Figs. 37-42.

becoming smaller and smaller and the point of exertion higher and higher until there is no remnant left showing an independent outline. The corresponding vascular bundle may, similarly, develop in some flowers (Figs. 37, 38) and be lacking in others (Fig. 39). The order of disappearance, as in *Verbena*, is variable, the bundle sometimes disappearing first, sometimes persisting after "loss" of the staminode. In those flowers in which it was present it in no case diverged into the exerted structure but continued upwards in the corolla tube, often giving rise finally to a branch system anastomosing with the adjacent petal lateral on either side. In *Saintpaulia ionantha* H. Wendl (Figs. 40-42) degeneration in the androecium has advanced further. The two postero-lateral staminodes range from outgrowths smaller than those in *Petrocosmea* (Fig. 41) to a scarcely defined "pimple" (Figs. 40, 42). The posterior staminode either appeared as a similar "pimple" or was no longer to be detected (Fig. 41). The corresponding vascular bundle sometimes gave rise to a considerable branch system (Figs. 40, 41), sometimes was completely wanting (Fig. 42), the order in which outward form and vascular system disappeared varying from flower to flower as in *Verbena* and *Petrocosmea*. In none of the flowers examined did the bundle of the postero-lateral staminodes enter the exerted

Figs. 37-39. *Petrocosmea nervosa* Craib. Three corollas from which the anterior petal and the greater part of the antero-lateral petals have been cut away, showing stages of degeneration leading to disappearance of the posterior staminode vascular system and of the exerted remnant which is non-vascular. Fig. 37. A corolla with a posterior staminode. The corresponding main bundle gives rise to a well-developed branch system. The bundles of the postero-lateral staminodes are differentiated throughout. Fig. 38. A corolla with posterior staminode and the corresponding vascular system less well developed than in Fig. 37. The distal portion of the bundle in the postero-lateral staminodes is undifferentiated. Fig. 39. A corolla in which the posterior staminode and the whole of the corresponding vascular system are lacking. The bundle of the left postero-lateral staminode is differentiated only in the lower portion, that of the right throughout. Figs. 40-42. *Saintpaulia ionantha* H. Wendl. Three corollas from which the three front petals and two stamens have been cut away, leaving the two postero-lateral and the staminodes which are all non-vascular. Fig. 40. The posterior staminode appears as a small "pimple." The corresponding main bundle has given rise to a well-developed branch system. Branch systems have also been formed by the main bundles of the two postero-lateral staminodes. Fig. 41. The posterior "pimple" has disappeared but the corresponding vascular system has remained well developed. The main bundle of the two postero-lateral staminodes is reduced to a short unbranched strand. Fig. 42. The posterior and right postero-lateral staminodes appear as "pimples" but the corresponding vascular systems have entirely disappeared. The left postero-lateral staminode is somewhat larger and the corresponding vascular system is well developed.

structure, *Saintpaulia*, in this respect presenting a contrast with *Petrocosmea*. In some specimens these bundles ceased at the level of exsertion and then remained unligified. In others they continued up in the corolla tube giving rise to a branch system. Lack of strict accord between "form expression" and vascular development is still more marked in the case of the posterior stamen member. A mere "pimple" indicating the exsertion point is sometimes to be found after all trace of the corresponding bundle has been lost (Fig. 42). On the other hand the bundle may persist and give rise to a well-developed branch system when no other indication of the "lost" member is discernible (Fig. 41). Or, again, "pimple" and bundle with branch system may both be present (Fig. 40). If in these two genera and in similar cases the main bundle of the disappearing stamen member should, at some penultimate stage of degeneration, give rise to lateral branches, the whole system remaining in the gamopetalous corolla, it is readily comprehensible that this system might persist unaffected by further diminution in size of a staminodal remnant already become non-vascular, or by its final disappearance.

ACANTHACEAE

In various genera of the Acanthaceae in which the posterior member of the androecium has undergone degeneration the corresponding bundle was found to persist, even after all other trace of the member had been "lost." This is to be seen in two species of *Ruellia*. In the flowers examined of *R. pulchella* Schott. and *R. formosa* Andr. there was neither trace of an exserted remnant of the posterior stamen nor any indication of the point of exsertion. Nevertheless the corresponding main bundle could still be traced for a considerable distance in the upper region of the corolla tube between the inner laterals of the midribs of the two postero-lateral petals.

SCROPHULARIACEAE (Figs. 43-50)

A parallel to the successive stages ranging from normal stamen to complete transformation into a normal "petal" with characteristic foliar venation, traceable in the phylogenetic history of the Ebenales-Primulales group of families, can be witnessed to-day in *Veronica*. In two unstable individuals, one *V. foliosa* var. *exaltata*, the other *V. longifolia* var. *rosea*, the following series was observed (Figs. 43-50):

(1) Normal flowers. Petals 4, stamens 2; staminal bundle unbranched (Fig. 43).

(2) Flowers in which the appearance is normal up to a point

about half-way between the corolla base and the natural exertion level of the stamen but in which at this point the stamen bundle gives off a pair of laterals, one passing into the petal on one side, the other anastomosing with the nearby lateral of the petal on the other side, the main bundle being prolonged for some distance above this point as a midvein in a ridge of thickened tissue which is not continued up as an exerted filament but shortly comes to an end (Fig. 49).

(3) Flowers in which one stamen has been "lost" but in which the bundle still persists (Fig. 44).

(4) Flowers in which one or both stamens show petaloid development in the region of the anther through prolongation of the connective (Fig. 45).

(5) Flowers in which an antherless staminal member appears as a petaloid structure with pinnate venation, exerted sometimes from the inner face of the corolla tube, sometimes from its basal margin, tubular below, becoming expanded above and split lengthwise, generally but not invariably, on the side turned towards the inner face of the corolla (Figs. 45, 49).

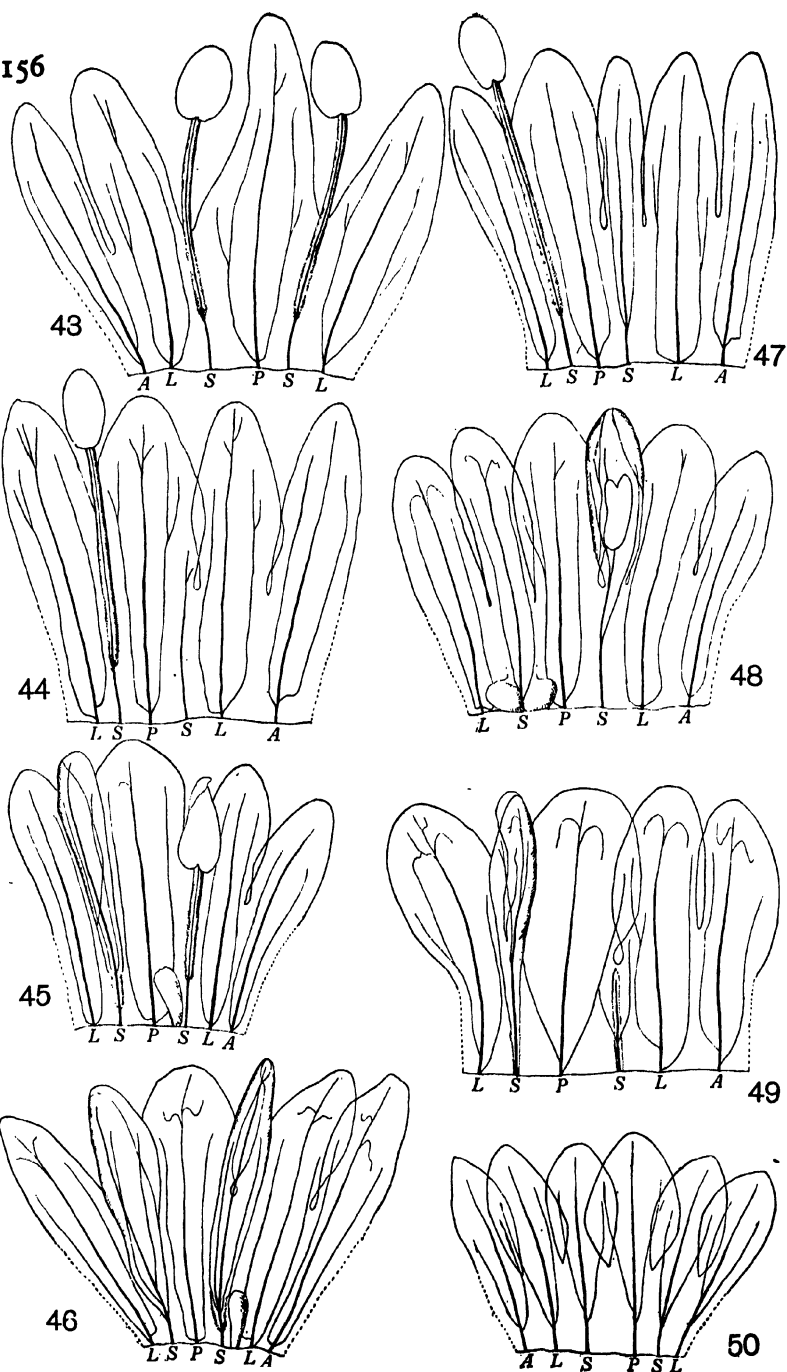
(6) Flowers in which both staminal members are antherless, petaloid, and folded or tubular (Fig. 46).

(7) Flowers in which the modification of the staminal member into a structure resembling the other petals is complete, the whole transformed member and its vascular system of midvein and two laterals lying in one plane, the basal portion of the member merging smoothly in the region of the tube with the petal on either side (Figs. 47, 48, 50). The corolla then appears to be five-petalled or six-petalled according as one or both stamens have reached this final stage¹. If such six-petalled corollas are detached it may be impossible to determine from their outward form which are the four original petal components of the corolla and which the two "petals" representing the transformed stamens². In such a modified *Veronica*

¹ Such five- and six-petalled flowers destitute of stamens are not to be confounded with those having five and six petals together with a normal androecium. These latter arise through reversion and duplication as described elsewhere (15). The vascular bundles which supply such corollas are those proper to the corolla whorl, whereas corollas of the former class receive bundles proper to the surviving members of the antesealous stamen whorl in addition to the midribs of the true petals. In the earlier stages of transformation from stamen to "petal" in *Veronica* the two transformed petaloid structures can be clearly seen to belong to a separate whorl within the corolla tube and attached to the basal margin but free from the surface.

² Identification is, however, generally possible here, and probably always in *Soldanella*, owing to the difference in level at which the lateral branches arise in the true petals as compared with the "staminal" petals.

156



Figs. 43-50.

flower we have the precise counterpart of the *Soldanella* type in Primulaceae. Here too, normal and "staminal" petals may be so similar as to leave identification by outward form uncertain without the presence of the surviving stamen whorl to give the clue. The difference between the two cases lies only in the fact that in *Veronica* the range from the (present-day) type to the end-result is accomplished in the course of the ontogeny of an individual, while *Soldanella* represents the same end-result in a race history which goes back so far that the type which begat the line of descent survives no longer. As yet we lack an appropriate system of terminology which will serve to distinguish a gamopetalous corolla composed entirely of original petals, such as characterises almost all flowering plants, from the normal primulaceous and the exceptional *Veronica* corollas in which "staminal" petals, often identical with the original petals, are interposed between the latter. This lack is due no doubt to the rarity of corollas of this latter class. Their rarity is due to the fact that in other dichlamydeous types in which only one staminal whorl is functional, it is usually the antesealous whorl which develops

Figs. 43-49. *Veronica foliosa* var. *exaltata*. Corollas of different composition split longitudinally and laid flat, viewed from the inner face. Fig. 43. From a normal flower (C4 A2). Fig. 44. From a flower in which one stamen is represented merely by its bundle (in the centre). Fig. 45. From a flower in which the right stamen shows a small petaloid expansion of the connective. At the base of the filament a small glandular flap of tissue. The left stamen has been transformed into a petal-like structure folded lengthwise in its upper portion. Fig. 46. From a flower in which both stamens have assumed a petal-like form. The left folded roughly in half lengthwise, the right with both margins reflexed and overlapping at the back. Beside it at its base a small glandular flap of tissue. Fig. 47. From a flower in which the right stamen (the central structure) has been completely transformed into a petal. Fig. 48. From a flower in which the left stamen is completely, and the right partially transformed into a petal. The latter member, which has formed an imperfect anther, is expanded above with both margins reflexed. At the base of the left petal-stamen a bilobed glandular flap of tissue. Fig. 49. From a flower in which the left stamen forms a structure tubular below, split and folded above. The right stamen is missing. Although there is no exerted structure the point of exertion is shown by the ending, just below the level of separation of the neighbouring petal lobes, of a ridge of tissue running upwards from the basal edge of the corolla. The corresponding main bundle which runs up in this ridge has given rise to a pair of laterals thus forming a system comparable with that found in a corresponding position in the predominant type of corolla in Primulaceae. Fig. 50. *V. longifolia* var. *rosea*. From a flower in which both stamens have been completely transformed into petal structures indistinguishable from the true petals. The members of the corolla and androecium are indicated by the letters below the corresponding bundles as follows: A, anterior petal; L, lateral petal; P, posterior petal; S, stamen.

normally. The degeneration or complete disappearance of the antepetalous whorl leaves the vascular system of the corolla unaffected; such a corolla consists of original petals only. That an appropriate terminology is still to seek is no ground for dismissing the facts in order to escape the difficulty. It would appear to be this difficulty which led Čelakovský to assert in reference to Klein's observations on the Cruciferae that the anatomical method carried to the extreme would land us in the *reductio ad absurdum* of regarding the marginal regions of one whorl as of the nature of another whorl(3). I have shown elsewhere that so far as the Cruciferae are concerned this dictum misses the mark, the relations of the vascular systems of the sepals and petals in this family being susceptible of a different explanation which, far from leading to a *reductio ad absurdum*, illustrates a mode of development of widespread occurrence—that of the commissural origin of the sepal marginals(13). On the other hand, as we see here in Primulaceae and in *Veronica*, and as I have shown previously to be the case in certain members of the Sterculiaceae(11) and in Thymelaeaceae, Penaeaceae and their allies(14), the situation which Čelakovský deemed impossible has been brought about. The "absurd" has become a reality. What we now need is to adjust our phraseology to meet this class of fact. Since, in her interpretation of the vascular anatomy of the Cruciferae A. Arber, after citing Čelakovský's *a priori* argument in support of her views, adds the remark that "the logical deduction from Klein's observations seems to be that vascular bundles are perfectly capable of transcending morphological boundaries" it is desirable to make clear that this deduction does not follow from the *facts*. Klein's figures of *Matthiola* and *Cheiranthus*, which are definitely stated to be schematic, are taken apparently from the fully developed flower(6). They show sepal marginal veins of commissural origin, which is correct, but the representation of the vascular strands in the gynoecium gives the appearance that the marginal veins of the valve carpels are derived from the main bundles of the consolidated carpels. This is not, in fact, normally the case. Examination of the young intact ovary in these genera reveals the four midribs of the four carpels unbranched and completely separate (13), p. 191, Figs. 10-12 and p. 193, Figs. 14, 15). As development proceeds, those of the fertile members give off in addition to the placental strands branches which run in the ovary wall. These latter strands mostly anastomose with the lateral branches derived from the valve carpel midribs, or they come to an end when they reach the radial sheet of thick-walled cells which

stands in line with each of the four external furrows marking the external boundaries between carpels (8), p. 453, Fig. 11). Here, the radial boundaries are also traceable in the internal tissues. But such delimitation is exceptional. In general, "boundaries," in any gamophyllous whorl, though a convenient conception, are without reality. We do not, then, find here the slightest evidence invalidating the reliability of the vascular ground-plan as a guide in the analysis of floral whorls.

Before leaving consideration of the Primulaceae and allied forms it will be instructive to glance for a moment at another family included in Primulales, the Plumbaginaceae. In this family, the five vascular systems proper to the petals alone are present in the corolla; there are no intervening bundles on the alternate radii. The reason is clear. In the members of this family, the marginal veins of the sepals are not commissural in origin but, when present, are derived by lateral branching from the sepal midrib bundles. There is an appreciable interval after the exit of the sepal bundles before the trunk cords consisting of petal midrib and antepetalous stamen bundle conjoined emerge on the alternate radii. Were strands for an antesepalous stamen whorl to be detached from the sepal midribs, these strands would arise either at too low a level, or too far outside the circle corresponding with the outer boundary of the corolla, to allow of their being incorporated with it. They would, of necessity, lie outside the corolla tube, an impossible arrangement. In the Primulaceae the sepal marginal veins are commissural. They carry out with them the midrib bundles for the petals. Sepal and petal midrib bundles emerge in quick succession. The surviving strands of the "lost" antesepalous stamens are detached from the sepal midribs almost simultaneously with the separation of the sepal marginals from the petal midribs, *hence petal midribs and antesepalous stamen strands come to lie on the same circle*. When calyx and corolla in due course become disjoined, the ring of the corolla tube consequently contains both sets of bundles. From the above facts we may infer that the antesepalous stamen whorl has been lost more recently in the Primulaceae than in the Plumbaginaceae, an inference which the existence to-day of remnants of the antesepalous stamen structures in a few genera in the former family entirely confirms.

With regard to the other particular case singled out by A. Arber, viz. Sargent's seedling of *Anemarrhena* (Liliaceae) showing two symmetrically placed, equivalent bundles in the cotyledonary organ, I think it cannot be questioned that here this organ was a double

structure, that is to say, that it was composed of two cotyledons fused together (7). Whether, however, Sargent's view (which A. Arber is not prepared to accept) that this condition indicates a derivation from a two-cotyledoned ancestor, is correct, cannot, I would suggest, be determined from a single specimen. Were this an isolated example it might well result from a variation in the individual ontogeny (deduplication) such as is frequently met with in floral whorls. Were it an occurrence of some frequency this fact would lend support to the suggestion of phylogenetic significance.

The remaining example cited by this writer as illustrating, in her view, an erroneous interpretation of the facts involving, again in her opinion, belief in the "doctrine of the conservatism of bundles" is that of the consolidated carpel. The term "doctrine" smacks somewhat of theory unsupported by concrete evidence. In so far its use here is misleading, for the observers whom this writer cites framed their interpretation to meet the facts which they found. In other ways also it appears to me that A. Arber's treatment of the question is somewhat unfortunate. The formulated conception of the consolidated carpel is expanded by her in the form of a (so-called) logical deduction. This logical deduction is then discussed and (supposedly) disproved. For example, on p. 232 this writer cites from my account a description of the "solid" carpel. She then adds the remark that this description "involves the belief that a carpellary leaf may be reduced to, its bundle *alone*," although it has been made abundantly clear in my accounts of the consolidated carpel that the boundary between neighbouring members, of whatever type, is judged to lie in the region between the limits of their respective vascular systems, or, if the systems become continuous, then along the line of contact. No reference is made by this writer to this fundamental conception regarding the delimitation of the members of a syncarpous gynoecium, but an extreme case is conceived and is then used as the basis of an argument which runs as follows. Logical deduction from the situation propounded involves belief in the survival of a bundle of a carpellary member "of which no external trace exists." But, asserts this writer (though incorrectly as I have already shown), there is no evidence of such survival, hence the theory of the consolidated carpel is (supposedly) disproved. To this argument it may be replied that the form of the premise is misleading, that the disputed evidence exists, and that consequently the conclusion to which the argument leads up falls to the ground. In my account of the gynoecium of certain liliaceous genera from which A. Arber quotes, I showed that

a series of grades can be traced from the obvious, well-developed carpellary member to one which has become reduced and, as I expressed it, "engulphed" by its neighbours on either side. We see, in fact, in *Fritillaria*, *Tulipa* and *Lilium* a series of stages in carpel reduction just as we see a series in stamen reduction in *Samolus*, *Deherainia* and *Steironema*. In the six-sided six-angled ovary of *Fritillaria imperialis* we have the clearest evidence, both from external form and from the vascular anatomy, of the presence of three sterile and three fertile carpels (9, p. 163, Fig. 76). In other species of *Fritillaria* (*F. meleager*, *F. armenica*), in *Tulipa* and in *Lilium* the three sterile carpels have undergone a reduction in size. From an expanded shape they have contracted to columnar form. How this has come about can be traced in the Garden Tulip, in which the fusion of the edges of two neighbouring fertile carpels over the internal face of each of the sterile members is not yet complete, as witnessed by the presence of a narrow cleft leading radially outwards from each loculus in line with each "engulphed" sterile carpel (9, p. 163, Fig. 78). Three longitudinal furrows on the external surface of the ovary in corresponding positions probably indicate a similar all-but-complete union of the *outer* edges of these same fertile members, thus bringing about the envelopment of the sterile members on their *outer* face as well¹. In *Lilium Martagon* the internal cleft is lacking, the line of fusion of the lateral faces of the fertile carpels is no longer traceable (9, p. 163, Fig. 80). That no indication of the lateral boundaries of the fertile carpels should be recognisable in the internal tissue in this species is not surprising, seeing that in the ovule-bearing region the sterile carpel midrib is so situated that contact followed by fusion of only *two* layers of ground tissue cells in addition to the one layer of epidermal cells is necessary for the envelopment of this bundle on its inner face, whereas in the Garden Tulip the sterile carpel midrib runs up in the ovary wall so far from the loculus that the lateral surfaces of the fertile carpels which close over its inner face extend to about 25 or 30 cells, and fusion has not come about. In this connection it will be well to emphasise that a visible demarcation of the radial boundaries between ancestrally contiguous carpels in a syncarpous ovary does not, as a rule, occur, and is no more to be expected than is an obvious boundary between the neighbouring petal members in a

¹ That these external furrows can be due to a sudden decrease in turgidity of the tissue on the radii of the sterile carpel midribs owing to the appearance of the loculi is unlikely, since in other liliaceous genera (e.g. *Polygonatum*) the external rounded contour is even and unbroken.

gamopetalous corolla. The clefts referred to above in *Tulipa* separate the lateral faces of carpels which were not, presumably, ancestrally in contact but which have been brought near together through a shortening of the radial dimension of the intervening member. Now since in each of the three above-mentioned genera there are six main bundles in the ovary, three on the radii of the sepals and three in line with the petals, it is clear that these six bundles have the same equivalence in all three, and that the ovaries are constructed upon the same plan. But in order to understand that plan the whole series must be surveyed. If it be admitted that the six-sided, six-winged ovary of *Fritillaria imperialis* is composed of six carpels, then, obviously, it is not possible to maintain that in the other liliaceous types mentioned above the three corresponding sterile carpels have not undergone reduction to the columns of tissue supplied by each of the three antesepalous carpel bundles, or to assert that there is no evidence that a floral member can be represented by such a unit of tissue. Yet though *Fritillaria* and *Tulipa* supply an essential clue to the nature of the liliaceous gynoecium, this writer, ignoring the concrete, builds up her argument from an imaginary case. Incidentally it may be added that where one set of carpel members becomes encircled by another set in the region of the ovary, as in the two types under consideration, it does not necessarily follow that the former may not exhibit "external form" in the region of the style or stigma.

From the considerations set out above it will be seen that A. Arber's line of argument in reference to the gynoecium is open to the same objection as her statements in regard to the primulaceous androecium. It is obviously necessary, where a series of grades occurs in the course of the process of degeneration, to take the whole series into account in order to arrive at a true interpretation of the lowest grade in the scale. In her presentment of her case this writer ignores the higher grades, and then does not hesitate to pronounce that the current interpretation of the final stages is entirely without foundation. Can *any* value be attached to assertions of this character?

In another respect it appears to me that in her discussion of the general question of the existence or non-existence of surviving bundles, this writer misses the fundamental point, and that through treating all cases of reduction *under one head* she is led to erroneous conclusions. When *independent* members of a floral whorl are in process of disappearing gradually, it is very usual for the last remnants of the members to persist after the corresponding vascular

bundles have been lost, as happens in the case of the fifth sepal in *Veronica* (15), p. 466, Fig. 41 b). The same order of disappearance may be seen in the bracts and calyx teeth of various species. When, however, the member which is disappearing as such is "lost" through being incorporated into another whorl, or when reduction in number is the outcome of fusion or failure to segment, the vascular bundles of the missing member frequently remain. The reason for this opposite result in the two classes of cases is obvious. When reduction in number occurs as the result of suppression of an *independent* member, the only destination available for the corresponding bundle pursuing its normal course is removed, and the bundle is not developed. When reduction in number takes place by a process of incorporation or fusion, the bundle can still pursue its original course in the tissue in which it lies. Examples of the loss and of the persistence of *stamen* bundles in these different circumstances have already been discussed at length in the preceding pages. Instances of the persistence of *perianth* bundles when reduction occurs through fusion and incorporation are furnished by the two following particularly clear and instructive cases. In *Veronica* the corolla shows reduction from the ancestral five-membered to a four-membered condition, through failure to become segmented in the mid-line at the back, in other words, through congenital fusion of the two postero-lateral petals. This process of reduction dates so far back that this condition has become characteristic of all but some four or five species in this large genus. Yet in the bulk of these species the two postero-lateral midribs still persist, giving rise to separate branch systems. Successive stages in the decline in development of the two systems leads eventually to the stable condition already reached in a minority of the species in which only one midrib giving rise to one system is present in the posterior segment (15), p. 488). In the leguminous plant *Saraca indica* the normal ten members of the calyx and corolla are represented by only four petaloid structures, but the ten vascular bundles of the normal perianth are all developed, two being present in each member of one pair of these structures and three in each member of the other pair (10), pp. 236-38, Figs. 30-32).

We have then incontestable proof that in some cases where reduction in number is brought about by fusion or incorporation with other structures the vascular bundle of "lost" members does persist. A. Arber's view to the contrary, though claiming to be based on an examination of the relevant evidence, is in fact founded upon argument drawn from an arbitrary selection of data. Apart from this,

however, it is difficult to understand how this writer reconciles her acceptance of Darwin's interpretation of the orchid flower with her pronouncement (*loc. cit.* p. 234) that "there seems indeed to be no escape from the conclusion that there is a complete absence of positive evidence for the vestigial survival of vascular tissue after the organ it supplied has ceased to exist."

At a later point, turning from evidence to speculation, this writer puts forward the following suggestions regarding the gynoecium: (1) That in carpels there has been a shift in vigour of vascular development from midrib to margins, and that consequently, as has been suggested by Hamshaw Thomas (18), the stigma of the traditional type of carpel may well be a double structure. Such a feature, she points out, would be compatible with the view which has been put forward by Eames and Wilson that "normally, in the majority of Angiosperm families, a carpel receives three traces, a dorsal or midrib bundle and two ventral or marginal bundles" (4, p. 253). (2) That some special relation (of what nature is not specified) may well exist between the carpels and the axis, since these members "not only are, but presumably always have been, the *ultimate* members borne by a shoot of limited growth."

With regard to the first series of suppositions it may be said that the idea that a transference of vigour has taken place in the carpel from the midrib to the margins becomes necessary on the traditional view that all carpels are of one uniform type. On the theory of carpel polymorphism it is superfluous, the *vigorous marginal* veins of the former theory being interpreted, in general, as the *main* bundles of intervening consolidated carpels. The idea that the carpel normally receives three traces and the conception of the duplex stigma in the valve type appear to be founded on the appearances seen in the acyclic apocarpous gynoecia of Ranunculaceae and Rosaceae. So far as I am aware, no indication of a twofold nature is to be seen in the stigmas in those types in these families having true monocarpellary ovaries, i.e. in those which ripen into the indehiscent achene. Such a twofold appearance appears to be confined to those ovaries which are classed as follicles, and among the follicles which I have investigated I have not yet come across any which appear to me to be composed of a single carpel. One important conclusion which emerges from the theory of carpel polymorphism is that the vascular supply of the valve carpel is *in its origin* precisely similar to that of the members of the other floral whorls. The essential point is that, typically, a single vascular strand leaves the central cylinder

to become the carpel midrib. Whether such a carpel "receives" one trace or three *at the level of exertion* is merely a question of whether the midrib happens to give off its first laterals before or after exertion, the former condition being quite exceptional, the latter characteristic of the great bulk of Dicotyledon types.

As to the second of the above suggestions, it is not only gratuitous but apparently inconsistent with the facts. If some special relation exists between carpels and axis due to these members being the last of the series of laterally-borne structures, then in the case of an elongated floral axis such as those of *Magnolia*, *Liriodendron* or *Myosurus* should we not be able to observe some difference between the first and last carpel members in a series of this length? But all, as they develop, resemble one another. Again, if this postulated exceptional relation were a reality, then should we not expect to see a difference between the members of the gynoeceium in a normal flower and those of a flower in which owing to proliferation they are followed by additional floral members? Yet in individual flowers of *Mecynopsis cambrica* and of *Cheiranthus Cheiri*, in which the members of a second flower are sometimes developed within the original ovary, the carpels of this latter ovary were found to be similar in number and position to those of the ovary in a normal flower.

Finally, this writer gives expression to the opinion, unnecessary, one might have supposed, in view of the fact that it must surely be held by all, that in investigations of carpel morphology it is desirable to study the flower as a whole and to take into account that rhythm in growth which underlies the whole floral ground-plan. Seeing, however, that this writer has considered such expression of opinion called for, it may be well to emphasise the following points. The theory of carpel polymorphism has been developed from a study both of the outward form and of the internal anatomy of the several floral whorls and of the axis which bears them. A survey of the whole of these data served to bring out the relationships of the vascular system with the external form. When these relationships are appreciated, it becomes possible to arrive at a more accurate analysis of the different whorls and a fuller comprehension of their interrelations than is possible from an external examination alone.

Furthermore, on this theory the conditions determining the position of the carpels are visualised as resulting from the rhythmic alternation of the whorls and the state of "block" or "congestion" increasing from without inwards often set up before the outer whorls are exerted. On this conception we are able to understand

the occurrence in the completely isomorous six-whorled Dicotyledon flower of antepetalous loculi and the resulting obdiplostemony.

We may conclude these comments with the following summary.

SUMMARY

1. The "loss" of a floral member may occur in at least three ways: by simple suppression, which is often a gradual process, by simple fusion with a neighbouring member, and by incorporation into another whorl.

2. Where a member is lost by simple suppression, the order in which outward form and vascular bundle disappear varies. In the case of an independent structure the bundle usually vanishes before the last remnant of outward form (e.g. fifth sepal in species of *Veronica*). In the case of degenerating members of an epipetalous androecium the bundle may persist, in whole or in part, after the corresponding member is no longer represented in any outward form (individual flowers of *Salvia schiedeana*, *S. rutilans*, *Ruellia pulchella*, *R. formosa*, *Petrocosmea nervosa*, *Saintpaulia ionantha*). Or, the bundle may be wholly lost before the last exerted remnant ceases to be formed (individual flowers of *Saintpaulia ionantha* and of species of *Verbena*).

3. In simple fusion the vascular bundles concerned generally persist after all trace of the union as regards outward form has been lost (e.g. the duplex posterior petal segment characteristic of most species of *Veronica*).

4. "Loss" by incorporation into another whorl is exemplified in the familiar case of the Orchid flower, and in *Saraca indica* where the typical calyx and corolla of the leguminous flower are represented by one whorl of four petaloid structures. In both cases the bundles of the incorporated members persist.

5. Incorporation, when accompanied by transformation of the "lost" member, either partial or complete, may be correspondingly partial or complete. The vascular system then generally persists (e.g. the antesealous stamen whorl in *Soldanella* and individual stamens in exceptional flowers of some varieties of *Veronica*).

6. Although no living members of the Primulaceae or the Theophrastaceae develop a *fertile* outer (antesealous) stamen whorl, the evidence available gives undeniable proof that, as generally held, present-day types have been derived from an ancestral stock in which two stamen whorls were present, and that the vascular strands which run near the margins of the petal lobes but which do not arise

from the midribs of these members represent the persisting portions of the vascular systems of an outer antesealous stamen whorl now "lost" as such, through processes, here traced in detail, of modification and incorporation, but still surviving in some Sapotaceae.

7. This modification process has been of the same nature in both families, viz. partial or complete transformation from a structure of stamen to one of petal form, accompanied by (a) a specific additional development in the corresponding vascular system, viz. the formation of a pair of lateral branches; and (b) incorporation of a part or of the whole of this system in the corolla. [In families, where a fertile antesealous stamen whorl is present, and where the bundles for this whorl are carried out conjoined with the sepal midribs, the stamen component, after it is detached, ordinarily passes *unbranched* into the filament.]

8. The genera *Samolus*, *Deherainia* and *Steironema* (2 Primulaceae, 1 Theophrastaceae), in which the outer (antesealous) stamen whorl is represented by distinct though modified structures, as well as by the correspondingly modified vascular systems, form a series in descending order leading to the final stage characteristic of the bulk of the remaining genera in the Primulaceae, in which no distinct structures survive, but in which, nevertheless, the corresponding main bundles as far as the branching point, and the lateral branch systems still persist in the tissue of the corolla.

9. Successive stages in loss of outward form exhibited by the above-mentioned genera are as follows:

Antesealous stamen whorl in the form of

- (a) long antherless vascular filaments: *Samolus*;
- (b) short conical vascular structures: *Deherainia*;
- (c) small flattened non-vascular structures: *Steironema*.

In (a) and (b) the main vein of the corresponding vascular system is prolonged into the exerted staminal structure, while the lateral branches remain behind in the corolla. In (c) the main vein is not prolonged above the level of branching, the whole system remaining in the tissue of the corolla, as in those genera in which these staminal structures have disappeared completely as such.

10. *Clavija* presumably diverged at some point before the end-stage was reached; the modified sterile stamen members showing an upward trend in development resulting in increased size of the exerted structures and a corresponding increased development in the portion of the vascular system supplying these structures.

11. The chain of evidence is completed by *Jacquinia* and *Soldanella*, in which the antesepalous stamens are replaced by structures, similar in outward form and colouring, and in their vascular scheme, to the true petals with which they are aligned and united below, thus giving rise to a "compound" corolla which is in reality composed of two floral whorls moulded into one.

12. The series represented in the Primulales by *Samolus*, *Jacquinia* and *Soldanella*, and the bulk of the Primulaceae finds its counterpart within the single family of the Sapotaceae in *Reptonia*, *Labatia* and *Chrysophyllum*.

13. The transformation of the members of the antesepalous stamen whorl into petaloid structures, indistinguishable in their most fully developed form from the true petals, and their incorporation into the corolla which has occurred in the phylogeny of the Primulales and Ebenales, can be paralleled in the ontogeny of the numerous exceptional flowers occurring in certain varieties of *Veronica*. Here, where unmodified flowers are to be found on the same individuals, every grade in the transformation process can be traced from the exerted unmodified functional stamen to structures indistinguishable from the true petals and incorporated with them into the corolla.

14. The above facts go to show that A. Arber's contention that there is no evidence in support of the view that vascular bundles can persist after the floral member which they supply has been "lost," or that the antesepalous bundles in the primulaceous corolla are not those of an antesepalous stamen whorl which has been "lost," is opposed to the evidence and cannot be maintained.

15. Two conceptions cited by the above writer in the course of her argument regarding the relations of the midrib and margins in the traditional type of carpel, viz. that this type of carpel is fundamentally a three-trace structure, and that such a carpel may have two stigmas, rest entirely, so far as appears, on the appearance seen in those ranunculaceous and rosaceous ovaries which ripen into follicles. On the theory of Carpel Polymorphism such ovaries are not interpreted as consisting of a single carpel, individual carpels of the valve type having always, so far as observation has yet gone, a single stigma. Whether a valve carpel shows one vein or three at exertion level has no particular significance, the number of veins at this level merely depending upon whether the midrib happens to give off its first laterals below the level of exertion or after exertion has taken place.

16. Since in the apocarpous gynoecium the last ovaries of a long

series at the top of an elongated axis, such as that of *Magnolia*, *Liriodendron* and *Myosurus*, are similar to those at the beginning of the series, and since proliferation of the axis into the locus of a syncarpous ovary with production of additional floral members (*Meconopsis cambrica*, *Cheiranthus Cheiri*) does not appear to affect the construction of the gynoecium, the above writer's suggestion that a special relation may exist between the carpels and the axis, owing to the fact that these members are, and always have been the last to be formed, appears to be not only gratuitous but contrary to the evidence.

The accompanying figures were drawn by Miss D. F. M. Pertz, to whom I wish to tender my grateful thanks.

REFERENCES

- (1) ARBER, AGNES. Floral anatomy and its morphological interpretation. *New Phytol.* **32**, 231-42. 1933.
- (2) BROWN, ROBERT. Some observations on the natural family of plants called Compositae. *Trans. Linn. Soc.* **12**, 76-142. 1818.
- (3) ČELAKOVSKÝ, L. J. Das Reductionsgesetz der Blüten. *Sitzungsber. d. k. Böhm. Gesellsch. d. Wiss. Math.-Nat. Cl.* **3**, 142 pp. 1895 for 1894.
- (4) EAMES, A. J. and WILSON, C. L. Carpel morphology in the Cruciferae. *Amer. Journ. Bot.* **15**, 251-70. 1928.
- (5) KERNER VON MARILAUN, A. *Pflanzenleben*, 2. Leipzig und Wien, 1891.
- (6) KLEIN, J. Der Bau der Cruciferenblüte auf anatomischer Grundlage. *Ber. d. Deutsch. Bot. Gesellsch.* **12**, 18-24. 1894.
- (7) SARGANT, ETHEL. A new type of transition from stem to root in the vascular system of seedlings. *Ann. Bot.* **14**, 633-8 with Plate 33. 1900.
- (8) SAUNDERS, E. R. A reversionary character in the stock (*Matthiola incana*), and its significance in regard to the structure and evolution of the gynoecium in the Rhoeadales, the Orchidaceae and other families. *Ann. Bot.* **37**, 451-82. 1923.
- (9) — On carpel polymorphism. I. *Ann. Bot.* **39**, 123-67. 1925.
- (10) — Illustrations of carpel polymorphism. IV. *New Phytol.* **28**, 225-58. 1929.
- (11) — On carpel polymorphism. IV. *Ann. Bot.* **45**, 91-110. 1931.
- (12) — On carpel polymorphism. V. *Ann. Bot.* **46**, 239-88. 1932.
- (13) — On some recent contributions and criticisms dealing with morphology in Angiosperms. *New Phytol.* **31**, 174-219. 1932.
- (14) — The cause of petaloid colouring in "apetalous" flowers. *Journ. Linn. Soc.* **49**, 199-218. 1933.
- (15) — A study of *Veronica* from the viewpoint of certain floral characters. *Journ. Linn. Soc. Botany*, **49**, 453-93. 1934.
- (16) THOMAS, H. HAMSHAW. The early evolution of the Angiosperms. *Ann. Bot.* **45**, 647-72. 1931.

POSTSCRIPTUM

At the time of sending this article to press I was unaware of the figure showing a portion of the corolla of *Soldanella pusilla* which appears in Wettstein's *Handbuch der systematischen Botanik*, 1901 (p. 407, abb. 400, Fig. 1). In this figure a single lacinia of the corolla, intended, it must be supposed to represent the appearance to be seen on *each* sepal radius, is indicated in the accompanying legend as a staminode. No further reference to the figure occurs in the text. It is not possible, therefore, to be certain what would have been Wettstein's interpretation of the *Soldanella* corolla with more than twenty laciniae. (As far as can be judged the illustrations of *whole* flowers of the three species *montana*, *alpina* and *pusilla* shown in abb. 16, p. 45 of the same work do not indicate in any flower a higher number than twenty.) Nor is it evident to what extent Wettstein appreciated the significance of the vascular scheme and its value in elucidating the various forms of the *Soldanella* corolla and the corollas of other members of the Primulales alliance. For the vascular system in the figure in question differs in an important particular from the normal. Of the main lateral veins there shown two are depicted as extending from the floral member in which they take their rise into a neighbouring member. Such a course in the case of primary laterals is not typical, these laterals running normally to the laciniae of the sector, whether that of petal or staminode, to which they belong. The absence of reference to this figure in the text is the more surprising in view of the opinion held by some in the past that the missing antesealous stamen whorl is represented in *Soldanella* by the scales present in the throat of the corolla in some species. It is scarcely necessary to add that such an interpretation is wholly irreconcilable with the facts, and that the thickenings and scale-like outgrowths at the region of transition from tube to limb in the corolla of many members of the Primulaceae are simply of the nature of a corona and were so regarded nearly a century ago by T. F. L. Nees von Esenbeck (*Gen. Pl. Florae Germanicae, Gamopetalae*, 1, 1845). If the close relation between the segmentation of the corolla and the vascular scheme had not been fully appreciated by Wettstein, it may be that *as regards the endings of the veins* his drawing is not a *precise* facsimile nor intended to be taken as holding good for the species in general.

REVIEWS

Plants and Human Economics. By RONALD GOOD, M.A. Pp. xii + 202, with 8 maps. Cambridge University Press. 1933. Price 5s.

All who have attempted to teach advanced systematic botany at schools or universities must have felt the need of some background against which to display the somewhat arid catalogue of facts which are inseparable from the subject. In modern times, when human economics are assuming such vast importance in the life of every community, no more suitable background can be chosen than the relationship between plants and man. In the present volume the author has succeeded in producing a short and readable survey of this aspect of botany which should be warmly welcomed alike by teachers and students.

There are five introductory chapters, including an admirable summary of the improvements in agriculture effected by modern scientific research, followed by seven chapters devoted to particular groups of economic plants, such as cereals, pulses, fibres, etc. The concluding chapter gives a brief summary of the economic history of Britain since the eighteenth century.

The author's style is, on the whole, good, though there are, perhaps, too many almost epigrammatic phrases which sacrifice clarity to effect in a way unsuited to a text book.

Valuable features of the book are the maps showing the distribution of economic crops, vegetation, and coal- and oil-fields throughout the world, and the systematic list of plants mentioned in the text. The volume is attractively printed and bound and is moderately priced at five shillings.

J. S. L. GILMOUR.

Virus Diseases of Plants. By JOHN GRAINGER. 7 × 5 in. Pp. viii + 104, 6 plates, 5 text-figures. Oxford University Press. 1934. Price 6s.

The aim of this small book has been to introduce to the student of plant pathology the phenomena associated with plant virus diseases. There are seven chapters arranged as follows. The first, consisting of only three pages, contains a short introduction and historical survey. In the second chapter the relation of a virus to its host plant is discussed, while chapter III is devoted to a consideration of some properties of viruses in extracted plant sap. Chapter IV deals with aspects of the relationship between insects and plant viruses. Chapter V describes the economic effects and measures of control, while chapters VI and VII deal respectively with the classification and description of virus diseases and some aspects of general experimental work. The book concludes with a list of 445 titles and a general index.

In such a small book as this, there are only 76 pages of subject-matter, it might have been better to have covered less ground and to have dealt with the more important aspects of the subject at rather greater length. For this reason the book will have a limited appeal. The practical horticulturist will not find much information upon the virus diseases themselves, while there is insufficient detail for the research worker.

There are six plates, all of them new, but with the exception of I and IV they hardly give the student a clear idea of the general appearance of virus-affected plants and a better illustration could surely have been given of potato leaf-roll.

For an elementary treatise the book is somewhat overweighted by its unclassified list of 445 titles which occupy 26 pages out of a total of 104.

The appearance of the book is excellent, the text is clear and readable and there are few misprints.

K. M. SMITH.

Chromosomes and Plant Breeding. By C. D. DARLINGTON, Ph.D., D.Sc., with a foreword by Sir DANIEL HALL, K.C.B., F.R.S. London: Macmillan and Co., Ltd. 1932. Pp. x+112, with 25 figures. Price 7s. 6d.

Dr Darlington's book is based on a series of articles in the *Gardener's Chronicle*. Its aim is to show how the recent advances in our knowledge of chromosomes may be of value to the breeder by interpreting his results and guiding his future work. It will undoubtedly be welcomed also by the students and non-specialists for whom the author's *Recent Advances in Cytology* was too tough a morsel, for it summarises much that was treated more comprehensively but not more lucidly in that volume. We lose, as irrelevant, an account of the more recondite characteristics of chiasmata, and we gain very considerably in readableness.

Introductory chapters on the Cell and Mitosis provide an excellent approach to the subject, but are marred by unnecessary pronouncements on such controversial matters as the significance of nucleoli and the stage at which chromosomes divide in somatic cells. The related fault of citing very inadequate evidence for widely held views, as where constancy of chromosome number is said to make it "evident that these chromosomes persist in the nucleus during the resting stage, although they cannot be seen," arises from the smallness of the book, but should have been avoided. It is unfortunate too, that the much reproduced figures of mitosis in *Allium*, from Bělař's *Grundlagen*, show neither the doubleness of the chromosomes at early stages, nor the chromomeres, two features emphasised in the text. The failure to show chromomeres becomes more important since they are also omitted in the diagram of mitosis on p. 9.

Chapters iv and v on Clones and Apomixis and on Fertilisation and Haploidy respectively are well placed after the description of mitosis and before that of meiosis, and contain some interesting information. There is then a brief treatment of meiosis and its genetic implications along lines made familiar by the author's original papers and earlier book; and a discussion of the qualitative differences between chromosomes, in which the idea of balance is introduced. In this connection the statement that "the hereditary properties of the plant are not so much the *sum* of all these units as their *product*" (reviewer's italics) is surely meaningless!

Variation is dealt with in a strictly cytological manner with a discussion of chromosome gain and loss, fragmentation, segmental interchange and ring formation, and with no mention of point mutation, which falls outside the scope of the book. There follow seven chapters on Polyploidy, constituting an admirable statement of the present situation, and packed with information which no person with a scientific interest in his garden can fail to find entertaining as well as instructive. Here he will discover the real nature of many well-known garden plants—*Aesculus carnea*, *Primula Kewensis*, the Campanula "Telham Beauty," the cultivated strawberry, the loganberry, the Laxtonberry, the potato. He will learn the constitution of his hyacinths and tulips, his apples, pears, plums and cherries, and will understand their reduced fertility and their failure to breed true. Going further afield he will appreciate the speculations as to the origin of cultivated cereals and of the hitherto mysterious *Spartina Townshendii*, and he will feel that the artificial raising of *Galeopsis Tetrahit*, of hexaploid *Phleum pratense* and of *Digitalis mertonensis* really do throw long awaited light on a mode of origin of new species of plants. These chapters should be read by all biologists who are not specialists in this field.

There are very few misprints, but the reference on p. 8 should be to Fig. 7, not to Fig. 8.

A. R. CLAPHAM.

THE NEW PHYTOLOGIST

VOL. XXXIII, No. 3

20 JUNE, 1934

THE NATURE AND ORIGIN OF THE STIGMA

A CONTRIBUTION TOWARDS A NEW MORPHOLOGICAL INTERPRETATION OF THE ANGIOSPERM FLOWER

By H. HAMSHAW THOMAS

(With 15 figures in the text)

HISTORY shows that in most branches of science generalisations which formerly passed as fundamental laws of nature have had to give place to very different and generally more complex concepts as the result of long-continued investigation. Gravitation, the gas laws and the concept of the atom are well-known examples of subjects in which the views of the earlier observers have been profoundly modified. It is strange therefore that in the field of plant morphology the concepts which Goethe put forward in 1790 remain to-day the basis of the morphological interpretation of the flower. This is still more remarkable since Goethe's views were wholly subjective or idealistic generalisations, and subsequent to their inception botanical thought has been revolutionised by the doctrine of evolution, while a very considerable store of information about ancient plants has been amassed by the palaeobotanists. It may be that during the last seventy years students of plant form have been so busy discovering and describing that they have had little time or inclination to reflect on the significance of their discoveries in relation to fundamental concepts, while recently the study of function has become more attractive than the study of form. We owe a considerable debt to Miss E. R. Saunders for reopening the subject of carpel morphology, and for showing that in many plants the vascular anatomy is not entirely consonant with the classical theory. Whether we agree with her interpretation of the facts or not, it is clear that no real progress can be made in morphology without a thorough discussion of the issues involved.

THE FOUNDATIONS OF A SYSTEM OF MORPHOLOGY

In a recent address⁽²⁵⁾ I have tried to indicate the reasons why it is necessary to-day to examine carefully the whole structure of our morphological system, and more especially its foundations. Such an examination shows that our ideas of the significance of floral structure, and, consequently, all modern systems of taxonomy, rest on one fundamental proposition, viz. that the different parts of the flower represent modified foliage leaves. The corollaries to this are:

(1) That the flower is a modified vegetative bud, and therefore the most primitive floral type is that which most nearly resembles a vegetative bud. The hypogynous flower is consequently held to be more primitive than the perigynous flower, and the spiral arrangement of parts is considered more primitive than the whorled arrangement. Further, that all the floral parts are referable to an original and continuous spiral sequence even though they now show no traces of such a regular sequence in their ontogeny¹.

(2) That the most primitive carpel type is that which in its mature condition most closely resembles an infolded vegetative leaf, viz. the young follicle, consequently that the pluriovulate condition and the terminal stigma preceded forms with one or few ovules and those in which the stigma is not apical.

(3) That no carpel or group of carpels can be regarded as primitively terminating the apex of the flower:

As has been already pointed out, the grounds for this view and therefore for its corollaries are very unsubstantial and obtain little or no support from the study of either fossil plants or of the flowerless plants, while the developmental work of Prof. Grégoire⁽⁹⁾ shows that there are essential differences between the development of the flower and of the vegetative bud. It thus becomes necessary either to produce fresh fundamental evidence or to set aside these theories, with all that was derived from them, as assumptions which cannot be proved, and to endeavour to build up a new system. It may be that some, if not all, of the old canons of floral morphology will eventually prove to be

¹ The consequences of such an assumption are illustrated in a recent contribution to the discussion of carpel morphology⁽²⁸⁾. In this paper Prof. McLean Thompson assumes a spiral succession of the structures we are accustomed to call the placentae of the carpels. The two placentae of one carpel, on this reasoning, are separated by an angular divergence of more than 360°, although in several of his contour drawings they appear to arise in equal pairs. The evidence for such an argument needs, however, a very full demonstration, and the mere numbering of primordia in diagrams affords a poor foundation for a far-reaching theory.

well founded, but, if so, they must be based on arguments differing radically from those which have been hitherto advanced.

At the outset we must do our utmost to avoid a subjective outlook, the relationships of the parts of different flowers must be viewed from the standpoint of their possible evolutionary origin and not merely as parts of a mental scheme¹. It is thus impossible to dissociate floral morphology from phylogeny, in spite of what may be urged to the contrary. The fact that in the past phylogenetic speculations have proved worthless may be evidence for the falsity of the morphological ideas on which they were based, rather than against the phyletic outlook.

In the second place we must consider the floral organs as interdependent parts of a whole living plant, whose reproduction depends on their efficiency.

Any system of morphology should comply with two essential requirements:

A. The postulates on which it is based should be exposed, so that their solidity may be at any time tested and assessed. At the present time no extensive generalisation on form and structure can be reached without taking much as reasonably probable though unprovable. It is hoped that future study will transform some of the necessary postulates into theorems based on evidence. The chief postulates of the new morphologist may be stated as follows. Let it be granted that:

(1) Vascular plants have evolved from simple ancestors by processes similar to those disclosed by modern investigators of genetics, and that the *general* structural features of the plant are inherited characters.

(2) The earliest differentiation of ancestral forms was that of reproductive from somatic cells.

(3) While non-adaptational mutations may arise and persist,

¹ It may be argued that there is little difference in practice between a subjective and an objective approach, but this is not true. The subjective treatment of morphology regards form as an intuitively apprehended concept (cf. Mrs Arber's recent characterisation⁽¹⁾ of the work of Prof. Troll), and consequently considerations of physiology and phylogeny have no practical significance. Crudely stated, the *Caltha* carpel is regarded as a folded leaf because it looks like one. The objective attitude takes the material of which the plant is built, and considers it in relation to the processes going on within it and to its environment. The psychological apprehension of form has no value or meaning comparable to the synthesis of evidence derived from other diverse sources. It may possess value if supported by such evidence, but must always be regarded with suspicion owing to the risk of an anthropomorphic interpretation.

those structural or physiological mutations which are advantageous to the race will tend to be selected.

These postulates are doubtless open to criticism but will be allowed by most botanists. There seem to be, however, differences of opinion as to the extent to which floral structures depend on hereditary characters. It has been stated that "the period of time over which each phase of flowering is spread, the rate of growth, and the form, stature and state of nutrition of the receptacle at each phase, will determine the number, form, stature and distribution of the emergences on the receptacle, and what occurs within them" (28), p. 67). The same author says later (p. 110): "Each form of flower is accepted as an individual expression of the problem of flowering, and the number, form, disposition and state of each emergence are held to follow the dictates of the problem." If these statements have any objective meaning, they seem to imply the belittlement of hereditary characters.

We have now to make another postulate which is more speculative.

(4) All known vascular plants have arisen from the same or similar ancestral stocks, have developed under comparable habitat conditions, and have a physical constitution which is essentially similar. Consequently a uniform system of morphological concepts should be applicable to all vascular plants.

This debatable idea is basal for the "New Morphology." It is derived from the observation that all vascular plants are built up from the same basic type of cell, with cellulose walls, a vacuolated protoplasmic lining, a nucleus of a definite character, chloroplasts and a comparable physiological mechanism. The living parenchyma, xylem elements, stomata, cuticle, etc., seem to be essentially similar in all green plants of the higher ranks, and it therefore seems reasonable to suppose that organic forms which have appeared in one group might at some time have appeared or be likely to appear in another widely separated group. The older morphologists attempted to apply the principles of Angiosperm form to the elucidation of the Pteridophyta, but some recent authors say that arguments founded on one group of plants are not applicable to an unrelated group. The present writer, however, maintains that the types of evolutionary change which seem on good evidence to have taken place in a group such as the ferns, may be expected to have occurred in the ancestral history of the Angiosperms. An analogy may illustrate the argument: the forms and structures of all steel bridges may be held to be comparable, and to be capable of arrange-

ment in one or more evolutionary series because their form is ultimately conditioned by the properties of the material from which they are built; stone bridges form a distinct series not really comparable. So the vascular plants are held to be comparable with each other, but not with the Fungi. The analogy reminds us that it is obviously impossible to describe or explain the structure of a simple plant in terms of a complex and more highly evolved form, and so considerable caution must be exercised. But it is doubtful whether there is any fundamental difference between using structures found in one group of Angiosperms to explain the structures seen in another group, and explaining angiospermic structures by reference to ferns or fossil plants such as the Caytoniales.

B. The second essential for a morphological system is that it must be applicable to all the known facts of structure and development. Structural features may vary in plasticity and importance, but none of them can be ruled out as possessing no morphological significance. The classical morphologists were concerned only with the external forms of mature organs, and with abnormalities, but in considering the carpel we have to consider the following features:

(1) The ontogenetic development of carpels; the structure of the primordia and their subsequent changes.

(2) The external forms of mature carpels (or ovary).

(3) The vascular system of the carpels and its relation to the vascular supply of the floral axis.

(4) The position, form and structure of the stigma and style.

(5) The structure and arrangement of the transmitting (pollen-tube conducting) tissue. The path of the pollen tube.

(6) Abnormalities.

(7) The results of hybridisation and experimental culture.

(8) The forms and positions of the ovules.

(9) The evidence from fossil plants.

Our aim should be the formulation of a scheme representing the possible evolution of the carpel which is in accord with the facts derived from an enquiry into each of the above-mentioned subjects, and at the same time is satisfactory from the standpoint of plant physiology. Just as the physicist or physiologist tries to explain his observed data by making certain assumptions, working out theoretical results of the assumptions, and comparing the results with his observed data, so the morphologist should attempt to sketch out a picture of a possible evolutionary series of changes, whose earlier stages would fit in with our available fossil evidence, and whose later

phases would agree in *all* respects with the different carpels which exist to-day.

In a previous paper I suggested a provisional picture of carpel evolution based on the Caytoniales and showed how this might elucidate certain peculiarities of the vascular system as well as the occurrence of forking stigmas and anatropous ovules⁽²⁴⁾. This picture was a first approximation and was criticised, probably with justice, on the ground that the suggested mode of evolution of the stigma was unintelligible⁽²⁹⁾. In consequence the stigmas of many flowering plants have been studied in order to form a truer appreciation of their structure and of the path taken by the pollen tubes. The recent detailed examination of a new group of Pteridosperms from the Southern Hemisphere⁽²⁶⁾ has contributed to our knowledge of some of the Gymnosperms which existed just before the appearance of the first known Angiosperms, while the work of Dr T. M. Harris⁽¹²⁾ has provided the first real link between angiospermy and gymnospermy. It is thus possible to present a second approximation, and to deal especially with the origin of the stigma and style. If the view here elaborated is correct we have but a short step now missing in the complete transition from the cupule-bearing branches of the Pteridosperms to the carpel of the flowering plant as indicated by actual historical evidence.

THE ORIGIN OF THE STIGMA FROM THE VIEWPOINT OF THE CLASSICAL THEORY

It seems natural to commence by assuming the truth of the view which we have inherited from our ancestors and which regards the carpel as derived by the infolding of a leaf bearing marginal ovules. If we adopt the objective attitude and think out the possible ways in which the carpel may have evolved from some such gymnospermous structure we are at once confronted with the problem of how, when and why did the stigma arise. Most botanists have carefully avoided these questions, but there are clearly three different ways in which the stigma might have appeared:

(1) We may suppose that the appearance of a glandular structure, later the stigma, at the tip of the supposed fertile leaf was the first step towards the closure of the carpel. The pollen grains might have germinated on this structure and the pollen tubes have miraculously found their way to the ovules, which presumably turned round and became anatropous. When the ovules were being fertilised habitually in this way the closure of the carpel could and did commence⁽²⁷⁾. Now

even if a glandular tip appeared on a megasporophyll and the pollen germinated on it, would it be physiologically possible for the pollen tubes to reach the ovules? If they grew over the open surface of the fertile leaf, which must have been fully exposed according to this view, they would surely become desiccated before they reached their goal. If they grew through the internal tissues, how did they find their way to the ovules?

(2) The second way would be the inrolling of the leaf margins before the evolution of pollination through the medium of a stigma. This would meet the objection of the probable desiccation of the pollen tube, but it involves a more serious difficulty. The initiation of an inrolling process before the formation of the stigma would bring about the sterilisation of a number of the ovules by screening them from the pollen grains, consequently a mutation in this direction would be liable to early extermination.

(3) While it seems rather unlikely that the appearance of the stigma and the inrolling of the leaf would occur simultaneously in a single mutation, there is the possibility that the closure of the leaf was preceded by the development of a marginal band of secretory cells spreading round the funicles of the ovules from the base to the apex of the carpellary leaves. The pollen grains may have begun to germinate on this and to fertilise the ovules in a chalazogamic manner. Once this process had been established the leaf might commence to close and the pollen tubes would have to grow farther and farther from their point of arrival in order to reach the ovules. This suggestion is much more in accord with the structural facts mentioned below, but it provides only a partial explanation of the varied features of carpel structure mentioned above, and is opposed by Grégoire's observations on the form of the carpel primordia.

On the whole, and apart from any morphological conclusions as to the evolutionary nature of the foliage leaf and the fertile branches, I think that the theory of the rolled leaf is too naïve and must be discarded unless at last some definite piece of evidence can be brought forward in its favour.

The interpretation of the carpel on the principles of the "New Morphology" discards entirely the classical concept of the fertile leaf; we no longer regard the young follicle as the primitive type. The whole carpel is deemed to have originated from a branched structure by evolutionary stages totally different from those passed by the foliage leaf, though both structures are regarded as originally derived from specialised branches of the thallus. The ovules are held to represent

original terminal structures, the placentae separate branches, and the carpel wall a cupular structure which is quite distinct in origin from a typical foliar structure.

A new theory as to the origin of the stigma has been advanced recently (28), p. 108) which departs yet further from the classical view. The primitive flower is regarded as possessing only vegetative emergences, stamens and stalked ovules borne on a crater-like receptacle. "In time the stamens on the crater rim were diverted from fertility and were the forerunners of styles. Glandulation or stigmatism was a phenomenon of their diversion, and expressed itself, as to-day, and as in the modern styles, on their distal portions." This view appears to have little objective foundation, and in the absence of physiological or structural details about the structures in question it cannot now be usefully discussed.

ON THE NATURE OF THE STIGMA OF LIVING PLANTS

In reconsidering *ab initio* the problem of the stigma we must first ascertain the path of the pollen tubes in modern plants. English and German writers on carpel morphology say little on this subject, apart from a few cases in which the last stage of the journey is through the chalaza. But much information already exists, though further research is clearly needed¹. Capus(6) in 1878 investigated the paths of the pollen tubes in many flowers and demonstrated the presence of a specialised tract of tissue which conducts the tubes from the stigmatic surface to the placentae. He reached the conclusion that the stigma is only the upper termination of the so-called conducting tissue of the style; Dr Harris has suggested to me that it would be preferable to call this "transmitting tissue" in order to avoid confusion with the vascular elements. Capus found that this tissue generally extends down to the level of the lowest ovule. He stated that its formation commences by differentiation of the fundamental parenchyma in the ovary, and this differentiation later extends to the style and stigma. In many ovaries we find papillate cells like those of the stigma, while in each carpel two adjacent centres of formation of the transmitting tissue can generally be distinguished. In the style the papillate form of the cells is usually lost and they form a continuous parenchyma distinguishable by its specialised walls and contents; these cells sometimes show marked changes during their development, their walls swell, probably becoming gelatinous and later assuming the

¹ A brief summary of the literature is given in Schnarf's "Embryologie der Angiospermen" (22), p. 282.

appearance of collenchyma, especially in the Compositae and other sympetalous families.

Later Guéguen (10, 11) confirmed most of Capus' observations in an extensive systematic study of the apetalous and gamopetalous families, and in 1918 the late Prof. Juel (14) recorded the distribution of this transmitting tissue in the chief genera of the Rosaceae. One would think that its occurrence and distribution would play an important part in discussions on carpel morphology, but it has scarcely been mentioned in recent publications.

From the work just described we seem justified in regarding the stigmatic surface as really a specialised part of the inner wall of the ovary which exhibits a very definite differentiation. This differentiation commences at the base of the ovary and extends upwards. In a number of apocarpous carpels of the Rosaceae and Ranunculaceae the specialised cells extend as a double band just inside of or along the ventral suture. When considered in relation to the growth of the pollen tube the differentiation of these cells cannot be regarded as a change initiated at the tip of the carpel and extending downwards, and in a number of cases, e.g. *Tournefortia*, *Cotoneaster* (see Fig. 9), the transmitting tissue passes by the top of the ovary and enters it only from the base.

We can scarcely avoid the conclusion that the stigma originated on the ventral side of the carpel, perhaps nearer to the base of the ovary than to the apex, and that the subsequent development of the style, or of the ovary wall, has gradually carried it upwards. Instead of being a simple terminal structure it appears to be composed of two adjacent lobes or parts, their contiguous facies being lined with glandular hairs, continuous with the transmitting strands below. These lobes may either be separate or united to form a grooved structure with the stigmatic surface lining the groove (see Figs. 4, 5). This is not entirely a new view, for in 1840 Robert Brown (5) wrote: "Each simple pistillum or carpel has necessarily two stigmata which are to be regarded not as terminal but lateral." He considered that the forms and positions of stigmas in all flowers could be explained on this view and quoted *Drimys* (*Tasmannia*) (cf. Figs. 1-4) as an obvious example illustrating his idea. It is strange that this generalisation has been so greatly neglected by

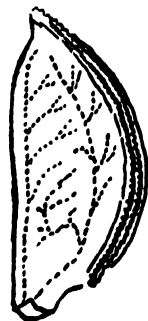
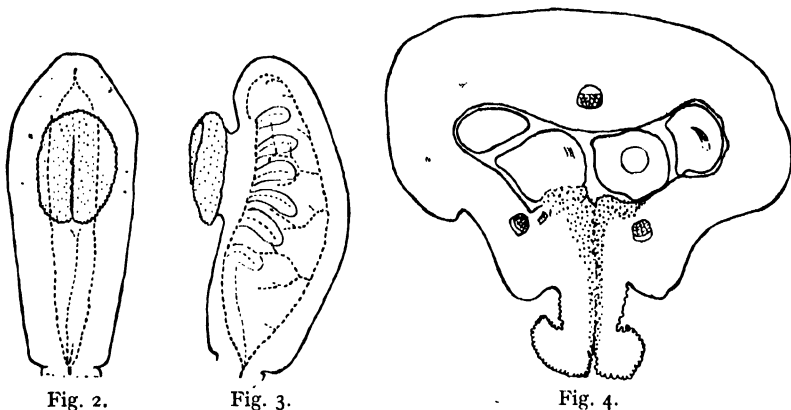


Fig. 1. *Drimys piperita* Hook. fil. Carpel showing stigma along ventral face and vascular system. $\times 12$.

subsequent authors, for Brown was at the height of his powers when this opinion was published, and he had given long and careful study to an immense variety of flowering plants.

We may find evidence of the double nature of the stigma in the many flowers in which it or its lobes fork into two equal halves, while the so-called gynobasic styles furnish good examples of the basal or lateral position of the organ which are difficult to explain on conventional lines.

The carpels of *Drimys* merit special attention, since this genus has been regarded as primitive on account of its vegetative structure. There is little doubt that the absence of vessels in the wood of



Figs. 2-4. *Drimys Winteri* Forst. Carpels from unopened flower, showing form and structure of stigma and venation. Fig. 2. Entire carpel seen from ventral side, showing placental bundles and fine branch between them below the stigma. Fig. 3. Cleared carpel in side view showing stigma, ovules, and veins (broken lines). Fig. 4. Transverse section of carpel passing through stigma, showing transmitting tissue (dotted), strong placental and dorsal veins and ovules. Figs. 2 and 3, $\times 18$; Fig. 4, $\times 54$.

several species may be taken as an indication of their antiquity. The form of the stigma in some of the Old World species (originally called *Tasmannia*) differs from that seen in the South American *Drimys Winteri* as shown in Figs. 1-3. In *D. piperita* Hook. fil. and *D. aromatica* F. Muell. the stigma forms a double band of papillate cells extending along the whole of the adaxial side of the carpel from top to bottom (see Figs. 1, 13 D). In transverse sections of *D. aromatica* it closely resembles the structure shown in Fig. 4. The secondary veins in the carpel wall arise from the placental bundles below and from a pair of strong dorsal bundles in the upper part of the carpel.

In *D. Winteri* the stigma is also a lateral double structure (Figs. 2-4). It is somewhat restricted in size, but as in the species mentioned

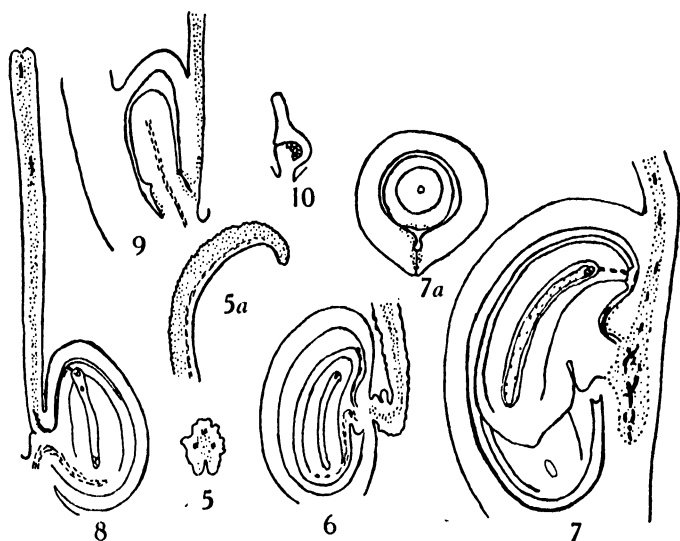
above it represents part of the margin, or rather the inner surface of the carpel wall. Transverse sections show that the delicate papillate cells which cover the stigmatic surface continue inwards through a central slit to the placentae within; this slit is the ventral suture and the carpel wall above and below the stigma is composed of perfectly uniform parenchymatous cells. All the ovules lie near the level of the stigma. The placental bundles are rather wide apart and in some specimens a slender branching vein lies between them (Fig. 2). The dorsal vein is here a single strand which curves over at the apex and gives off secondary veins into the carpel wall.

The other members of the Magnoliales appear to have stigmas referable to the types seen in *Drimys* (cf. Fig. 13). In *Schizandra* there is an elongated papillate structure with a central groove running along the greater part of the ventral side. *Trochodendron*, which also possesses wood without vessels, is essentially similar but the stigmatic surfaces are limited to the apices of the carpels which form a partly fused whorl; although the receptive area does not extend far down the ventral side, a fissure lined with transmitting cells continues to the base as in *D. aromatica*. No suitable material of *Illicium*, *Kadsura* or *Tetracentron* has yet been available but published figures suggest their similarity. The elongated apical stigma of *Magnolia* appears at first sight distinct from the *Drimys* type but is certainly linked with it through *Trochodendron* and *Schizandra*.

Juel's description and figures of the carpels of the chief genera of the Rosaceae at the time of fertilisation, with special reference to the structure of the stigma and style, the courses of the transmitting tissue and vascular bundles, the form and positions of the ovules, and the paths of the pollen tubes, provide the most complete evidence yet available (14). Although tied by the classical view of the carpel, Juel pointed out several features on which a totally different arrangement of the genera might be based by reference to the stigma and style. He was forced to exclude the Kerrioideae, which may now be held to be the most primitive forms, from his scheme of phyletic relationships; and, indeed, it is difficult to derive these forms from an ancestral type similar to the Quillajeae, though the reverse process is a simple conception.

The stigma in the Rosaceae, with the exception of *Alchemilla*, shows a ventral groove lined with transmitting tissue which corresponds to the ventral suture below; during its passage down the style the transmitting tissue generally becomes enclosed in the centre and

ultimately spreads out again on the inner wall of the ovary and around the placenta, lining the inner sides of the ventral suture. But in several genera, e.g. *Rhodotypus* (Figs. 7, 7a), *Coleogyne* (Figs. 5, 6), *Comarum* (Fig. 8), *Cotoneaster* (Fig. 9), *Cerocarpus*, *Waldsteinia*, *Geum* and *Alchemilla*, the transmitting tissue runs down to a point well below the top of the ovarian cavity before opening out, and in most of these cases Juel found the pollen tubes running down to the level



Figs. 5, 5a, 6. *Coleogyne ramosissima*. Figs. 5, 5a. Transverse and longitudinal sections of stigma. Fig. 6. Longitudinal sections of ovary. $\times 10$.

Figs. 7, 7a. *Rhodotypus Kerrioides*. Fig. 7. Longitudinal section of ovary. $\times 20$. Fig. 7a. Transverse section. $\times 15$.

Fig. 8. *Comarum palustre*. Longitudinal section of carpel. $\times 30$.

Fig. 9. *Cotoneaster integerrima*. Longitudinal section of ovary. Note distribution of transmitting tissue. $\times 13$.

Figs. 5-9 after Juel (1918). Transmitting tissue dotted.

Fig. 10. Young developing carpel of *Alchemilla arvensis* showing carpel wall with rudimentary stigma growing over the young ovule (after Murbeck).

of the ventral suture even though they afterwards had to turn round and grow upwards again to reach the micropyles (Fig. 7). In *Coleogyne* and *Comarum* (Figs. 6, 8) the styles are inserted laterally, and, as viewed in longitudinal section, appear as though the ovary wall had originated in ontogeny below the ovule, had curved round it, and, after completely enclosing it, had continued to grow upwards. In such forms as *Rhodotypus* and *Cotoneaster* there is no longer a gap between the dorsal side of the style and the ventral side of the ovary, and this ontogenetic fusion may have led to types like *Dryas* and

Waldsteinia, where the style appears to arise from the top of the ovary; elongation of an ovary of this type would give the *Kageneckia* type, which Juel thought the most primitive.

The present distribution of the members of the Kerrioideae suggests an argument in favour of their antiquity. In this group Juel includes four monotypic genera, *Rhodotypus* confined to Japan, *Kerria* to China, *Neviusia*, an endemic in Alabama, and *Coleogyne*, a Californian endemic.

Murbeck's studies (18) of the development of the carpel in *Alchemilla* showed that the carpel wall does actually grow in the way suggested above (Fig. 10), spreading round the developing ovule in what may loosely be termed a circinate fashion and finally closing at the base of the style.

A very cursory glance through botanical literature shows that the type of carpel development seen in *Alchemilla* is by no means exceptional and may even prove to be the more general type. Payer's figures of *Coriaria* and *Ailanthus* reproduced in Goebel's *Organography* (8) are well known, and many other of his figures (20) seem to show similar features. Quite recently the same mode of growth has been illustrated from *Boerhaavia diffusa* by Maheshwari (17) and *B. repanda* (Nyctaginaceae) by Bhargava (4), see Fig. 11, and the figures of *Lyonothamnus floribundus* given by Juliano (15) suggest the same type of development. It may well be found elsewhere¹, but details like this, which are not in accordance with the accepted views of morphology, are sometimes passed over without record.

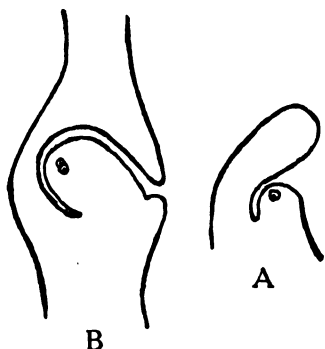


Fig. 11 A, B. *Boerhaavia repanda*. Two stages in the development of the carpel (after Bhargava). Note position of origin of the carpel wall and mode of closure. $\times 160$.

A SUGGESTED ORIGIN OF THE STIGMA

The question now arises: Can these facts about the stigma, transmitting tissue, style and ovary wall be explained or fitted into a morphological scheme without any far-reaching assumptions? Fortunately we are now able to outline a possible history of the evolution of the carpel which would co-ordinate the varied facts of

¹ E.g. *Ranunculus* as described by Troll ((29), Abb. 95) and by Salisbury ((21), Fig. 15); *Rivina* described by Joshi and Rao (13).

structure and combine them with what we know of fossil seed plants. One may call it a history rather than a phantasy because it commences with a group of plants which actually existed, and is based on the comparison of the plants of successive ages. Instead of having to postulate not only the structure of the supposed ancestral megasporophyll but also a series of evolutionary changes, we have only to grant that the study of the changes known to have occurred in a great group of plants which resulted in the production of the angiospermous habit, may be useful in explaining the evolution of the flowering plants.

It is not unreasonable to suppose that the flowering plants which have only been dominant in the world for a period of about one hundred million years were derived from the pteridosperms, which had previously existed all over the world for at least twice as long a period, if it can be shown that there is a possibility of deriving the fertile and vegetative structures of the former from those of the latter.

The ovules of the Pteridospermae were essentially terminal structures more or less surrounded by a cupule and borne on a branching structure (Fig. 12 A-F). During the Palaeozoic period the ovule-bearing structures were often associated in various ways with photosynthetic fronds, but in the known Triassic members of the group (Fig. 12 D-F) no expanded lamina was present though minute bracts and bracteoles occurred on the branches. As compared with the Palaeozoic forms these fertile branches were very much reduced, the ovules were produced in pairs, and in several examples the cupules of the terminal pairs were more or less concrescent (Fig. 12 F). The cupules had a recurved form, and in one genus the inside of the cupule was thickly lined with hairs. A little later, in the Liassic period, structures of a similar external form (Caytoniales, Fig. 12 G, H) passed from the gymnospermous to the angiospermous mode of fertilisation; instead of entering the micropyles the pollen germinated on the recurved tips of the hairy cupule and the ovary became closed. Here we see a lateral stigma which is part of the inner surface of the ovary wall. If we now assume that a similar process went on in the case of cupules which were concrescent, we should have a structure which is in all essentials an angiospermous carpel (Fig. 12 I, J). The original ovule-bearing stalks on this view would have now become the two adjacent placentae, the cupules growing from these stalks and curving over the ovules in three dimensions might have become completely fused except for their recurved papillate tips which form a

pair of lateral stigmatic surfaces, and since the whole structure is reduced from a much longer and more branched type, it seems possible that the concrescence of the cupules on the dorsal side might some-

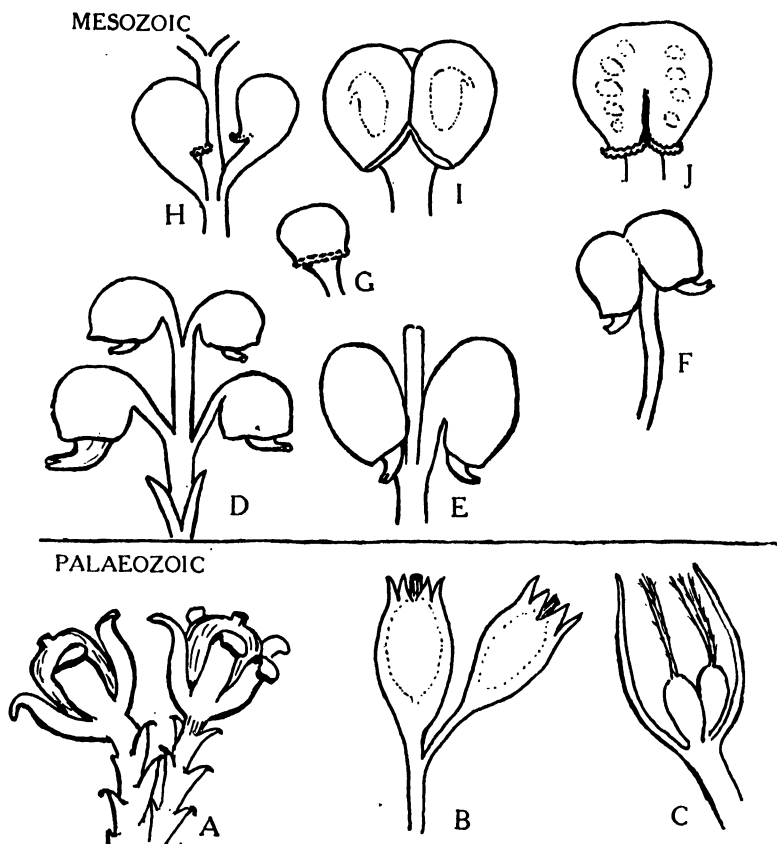


Fig. 12. Seed-bearing structures of Pteridospermae and Caytoniales suggesting a possible mode of evolution of a hypothetical carpel (J) with a gynobasic stigma. A, *Lagenostoma Lomaxi*. B, *Lagenostoma Sinclairi*. C, *Gnetopsis elliptica* (section). D, E, *Pilophorosperma* spp. Somewhat diagrammatic to show long and short pedicels. F, *Pilophorosperma geminatum* showing partly fused cupules. G, *Caytonia thomasi*, single ovary with broad basal stigma. H, *Gristhorpia Nathorsti*, pair of closed ovaries with basal stigmas. I, hypothetical form in which the numerous open seed-bearing cupules on a fertile branch have been reduced to a partly fused pair. J, hypothetical carpel in which cupules like those suggested in the previous figure have become closed with the formation of a basal stigma.

times include the last traces of the stem which formerly grew up between them (with the remains of its vascular supply). According to the relative rates of curvature of the cupule in the different planes,

the stigma should appear either towards the base of the carpel or in some other position along the ventral side (Fig. 13 A, D). It is likely that these ovulate structures were grouped together at the apex of a stem. But since the chances of fertilisation would be increased by the elongation of the stigma or its appearance at the top of the carpel, especially where the ovaries were crowded together, mutations in the direction of style formation would probably be selected, and so we arrive at a type of structure represented not only among the Rosaceae, but also in *Trochodendron*, *Magnolia* and a number of plants of various families.

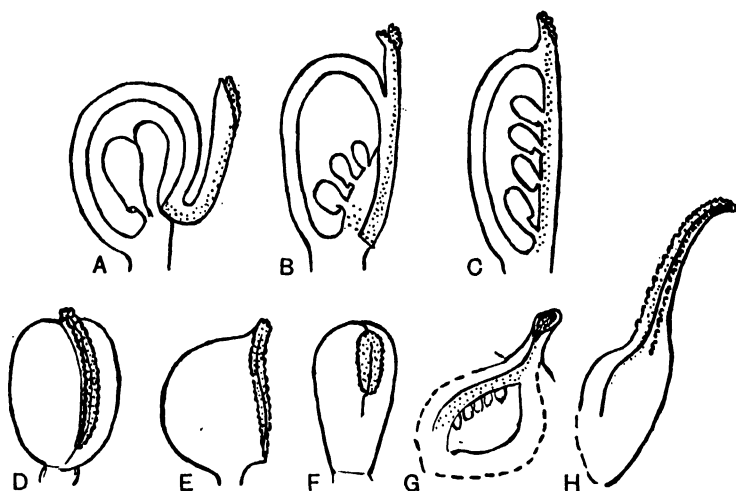


Fig. 13. Diagrams indicating two possible modes of origin of the apical stigma. A-C, series suggested by comparison of the carpels of the Rosaceae. D-H, series suggested by the Magnoliales. D, *Drimys aromatica*. E, *Schizandra*. F, *D. Winteri*. G, *Trochodendron*. H, *Magnolia*.

This upward growth of the stigma would leave a groove lined with the specialised cells which now form the transmitting tissue. The groove corresponds to the ventral suture, which in the Ranunculaceae, Rosaceae and Leguminosae often shows an actual slit at the base, as our theory would require. From this primitive carpel all the other forms can be very readily derived without great assumptions (see Figs. 13, 14). Concrescence or fusion of adjacent carpels gives the normal type with axile placentation; the young follicle with its terminal stigma would come by elongation accompanied by reduction of the apical curvature. Ovaries with free central placentation may have been formed when the constituent carpels were so close together that it was

impossible for the circinate cupules in closing over to come into contact with their respective placentae.

I believe that this theory is worthy of serious consideration not only because it is possible from the physiological standpoint, but also because it explains most of the important features specified above and which classical morphologists have often ignored or glossed over. It not only agrees with the ontogenetic observations mentioned earlier but with the observations of Goebel (8), Figs. 359, 364) that in the early stages of carpel development three centres of development may be distinguished. One may correspond to each placenta and one to the fused cupules. Grégoire(9) attaches considerable significance to the fact that the two placentae of the carpel are from the first

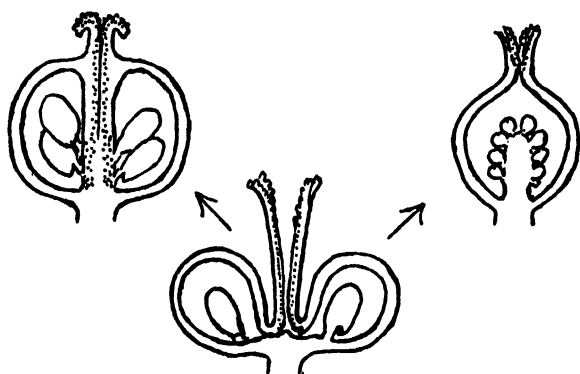


Fig. 14. Diagrams indicating suggested relations between ovaries with central basal styles (? primitive type), those with axile placentae (left) and those with free central placentation (right).

separated by a V-shaped slit and not by a U-shaped depression, which would be required on the old view. Our suppositions would be in accord with his observations. They would also explain the observations made on the Scitamineae(28).

It is probably possible to account for the various external forms of ovaries on any theory, but the new views suggest that the different types now extant may be the results of variations in the rates of growth and concrescence of the original parts, accompanied by modifications of a less extensive character than that required by the classical views. Our postulated primitive structure is probably nearer than the young follicle of *Caltha* to the average type of modern carpel. The different forms of stigmas, especially the so-called commissural stigmas, now seem readily explicable. The arguments with reference to the vascular system remain essentially the same as those

brought forward in my previous paper, and it may be noted that our new theory would lead us to expect the presence of the structures which Miss Saunders interprets as the fusion of $\frac{1}{2}$, 1, $\frac{1}{2}$ carpels. It is necessary in view of recent criticism⁽¹⁾ to reiterate my view that the general pattern of the vascular system must at present be regarded as an hereditary character, for variations in the size and development of the ventral and dorsal strands have been observed to occur without greatly affecting the general pattern. Such different types of venation as those seen in *Drimys* and *Caltha* may be explicable on the view that the carpel wall is derived from fused cupules.

I have not yet seen any phyletic explanation of the distribution of the transmitting tissue, and I hope that the investigation and discussion of this important constituent of the carpel may be stimulated by the views suggested above. The possible significance of the anatropous ovule was referred to in 1931, and the argument still holds, although neither the *Corytospermaceae* nor the *Caytoniales* have been found to possess ovules which could be termed anatropous.

It is to be hoped that future research may tell us more about the nature and significance of abnormalities, but the production of abnormal open carpels described by Lamprecht in *Pisum*⁽¹⁶⁾ suggests possibilities in this direction. The fact that open leaf-like carpels may be produced by crossing may be taken to suggest that the abnormalities on which the classical morphologists have relied so much should not be regarded as reversions, but as new types of structure resulting from a rearrangement of genes.

However, a large number of widely different types of abnormal carpels have been described, and it seems reasonable to regard plants as capable of varying in many directions owing to changes in the nuclear or physiological constitution of their meristems. The problem which confronts us is whether these structural variations arise at random, or whether they are related to the fundamental morphological construction of the organ. Is it possible to conceive of all the different abnormal carpels as variations in every possible direction from one central type of structure? If so, while no single form might be truly atavistic, the whole series might have considerable morphological value. It may be that the present concept of the carpel as originally composed of four or five distinct parts (two placentae and two cupules, sometimes accompanied by the relics of the stalk between the cupules), which may be fused or separated, hypertrophied or reduced in a number of different ways, provides such a central type of structure.

THE EVIDENCE FROM FOSSIL PLANTS

Some authors deprecate the introduction into questions of Angiosperm morphology of ideas derived from the study of fossil plants. They have perhaps overlooked the significance of the fossil record in morphological studies. When we take a survey of a problem like that of the carpel, we find different authors commencing with different sets of basic assumptions; they examine one or two aspects of the modern structures and reach divergent conclusions. Each conclusion may be valid by reference to the assumptions and to the evidence employed, but how are we to decide which is nearest to the truth? The decision often rests with the student of fossil plants. Unless the Angiosperms were separately created, or evolved from distinct unicellular ancestors through forms of which we have no records, their history and morphology must be interpreted by reference to ancient plants. Few people realise how much we know of the development of plant form from the Devonian period to the present day. It is only the adherence to a discredited system of morphological concepts that transforms the fossil record into a mass of unconnected fragments of plant history.

The view of the carpel outlined above not only seems to fit the facts derived from the study of modern plants but it also links on to the chronological series of fossil plants, which suggests that the changes indicated may have actually taken place, though the known fossils are to be regarded as successive members of a race rather than as a single genetic series¹.

As some of these fossils are not yet well known we may now add some further reference to them. Commencing with the well-known Upper Palaeozoic *Lagenostoma lomaxi* (Fig. 12 A) we have a species with a very open and deeply divided cupule which had glandular hairs on its inside (19). Arber described another species, *L. Sinclairi* (2), in which the cupule was larger and more campanulate, only lobed near its margin (Fig. 12 B). In *Gnetopsis* (19) there were several seeds with long, micropylar structures completely surrounded by a two-valved cupule (Fig. 12 C), on the inside of which Oliver and Salisbury found hairs. *Pilophorosperma* (26), a form which probably had a wide distribution in the Southern Hemisphere in Triassic times, had single

¹ Special attention must be drawn to the method by which the fossil forms are arranged in a phyletic series. The species mentioned show different organisation levels, or stages in a series of changes. By the abstraction of the relevant characters it may be said that the *Caytonia* type of structure may be derived from the *Lagenostoma* type, though there is no reason to suppose that the genus *Caytonia* was derived from a *Lagenostoma*.

seeds enclosed in recurved cupules thickly lined with hairs (Fig. 12 D, E); some of the hairs were long while others were probably glandular. These cupules were usually produced in opposite pairs and were often borne on pedicels, though in certain species they were sessile. In *P. geminatum* the terminal pairs of sessile cupules were more or less fused (Fig. 12 F), while an example of another species shows that the cupules arose in close contact though separating later. It should be noted that all these objects were of small size. The mature cupules were less than 4 mm. across, while the whole branched structure, bearing perhaps eight cupules, was little more than 2 cm. long. They were true Gymnosperms, the curved micropyles projected from the cupules and probably absorbed the pollen grains by a drop mechanism.

Dr Harris⁽¹²⁾ has recently described a species from Greenland which is of the greatest morphological interest. It is the earliest known member of the Caytoniales and had an ovary which became closed at an early stage in its development leaving a flange which closely resembled a stigma. But the ovules in this form were undoubtedly fertilised in a gymnospermous way, since in more than half of the many seeds examined a number of pollen grains were found in the micropyles. Dr Harris had reasons to suppose that the micropyles were at an early stage connected with the "stigmatic" opening by canals, and he suggested that the inside of the fruit wall was very thick and fleshy, embedding the seeds in pockets and nearly obliterating the cavity of the ovary. After studying the *Corytospermaceae* it seems reasonable to suggest that the tissue in which the seeds were embedded may have been composed of a mass of more or less fleshy hairs, growing from the ovary wall, and perhaps forming a pseudo-parenchymatous tissue, something like the flesh of the orange. There can be little doubt that these plants which Harris calls *Caytonia thomasi* are closely related to the Yorkshire members of the Caytoniales, though the propriety of including them in the genus *Caytonia* is debatable¹.

The mode of fertilisation of this new species naturally raises the question as to whether *Caytonia Sewardi* and *Grithoropia Nathorsti* from the Jurassic⁽²³⁾ were really angiospermous after all, but there can be little doubt on this point. Before these forms were described more than 150 seeds were macerated and studied by the same method

¹ The difficulty is one, familiar to palaeontologists, of deciding on the best nomenclature for an early ancestral form and two later types probably derived from it.

used for the Greenland forms. A pollen grain occurred in one seed only, and since this was a mature seed lying free in a matrix full of similar microspores no significance could be attached to it. In complete fruits pollen grains were only found on the young stigmas, and these stigmas represented a recurved portion of the ovary wall, thickly lined with papillae on its inner side, and precisely similar in form and structure (so far as it is observable) to one of the stigmatic flaps of *Drimys* (see Fig. 15). No doubt the ovary of these Jurassic forms was open at a very early stage in its development just as in the

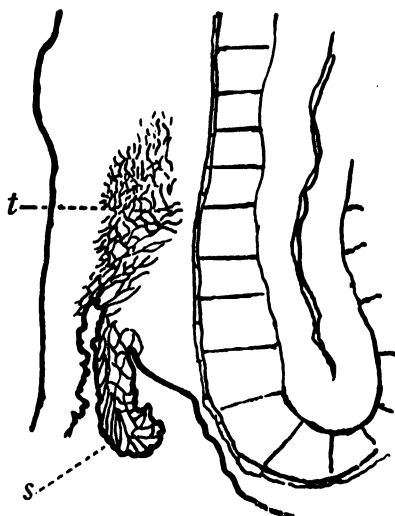


Fig. 15. Section through fruit of *Caytonia Sewardi* showing stigma (s), and internal cutinised cells (t) which may represent the transmitting tissue. Part of one of the seeds is shown. $\times 109$.

case of the carpel, but it must have become closed up very soon by the growth of the papillate hairs of the stigmatic surface.

It may be mentioned here that Prof. John Walton has recently discovered a very young leaf of *Sagenopteris* which, owing to its hook-like form, not unlike that of young leaves of *Vicia faba*, provides a further reason for regarding this type as allied to the Angiosperms rather than to the ferns.

The study of the Corystospermaceae and the Caytoniales has not yet provided any definite information upon the question of the aggregation of seed-bearing structures to give what we call a flower. The consideration of this subject must be deferred pending the collection of further facts and the study of newly acquired fossil

evidence. It may be noticed, however, that there is good evidence from all groups of the seed plants of a tendency for the fertile parts of the plants to become aggregated together in compact clusters at the tips of the ultimate branches. There is thus no reason why the value of the fossil forms just mentioned should be discounted on the ground that they did not bear flowers.

Although the Caytoniales probably overlapped the flowering plants in point of time, we have yet no certain evidence from periods before the Upper Cretaceous on which we can base any opinions with reference to the forms of carpels and stigmas in the earliest flowers. Hitherto no attempt has been made to deduce the probable characters of the more primitive types from the study of Cretaceous and Early Tertiary angiosperms, for it has been generally agreed that such forms had already become highly specialised, and could in no sense be regarded as primitive. We must notice, however, that this opinion is a further consequence of the classical views of morphology. It is only a preconception of the nature of the primitive flower which has led botanists to hold that the flora which existed some ninety million years ago was as highly evolved as that of the present day. It is very probable that the "primitive flowers" for which we have searched in vain so long, and whose discovery had to be awaited for the solution of all the problems of angiosperm morphology and phylogeny, are chimeras of the imagination. We can find no traces of them because they have never existed.

If we set aside the classical theory as discredited, and concede that different groups of Angiosperms may have arisen along parallel lines from distinct groups of pteridosperms, we may come to regard the Upper Cretaceous and Eocene floras in quite a different light. Why should we not accept the evidence at its face value, and try to ascertain the probable features of the more primitive flowers by an analysis of the features of those present-day groups which were certainly present in these early floras? This is a matter for further study, but a few examples may be given which suggest that such a study may have a bearing on the subject of this paper. In the Upper Cretaceous flora of South Carolina which was described by Berry(3) the Urticales seem to form one of the most abundant orders, and most palaeobotanists have agreed that plants allied to the modern *Ficus* were widespread at the time. Now the carpels of *Ficus* usually have a lateral style, while those of *Dorstenia Poggei* figured by Fries(7) are exactly of the type which was suggested above as being one of the most primitive forms, the style arising near the base of the ovary and

forking above into two stigmatic arms. *Artocarpus*, another genus which has existed since Cretaceous times, appears to have somewhat similar carpels. Berry states⁽³⁾ that magnolias are common everywhere in the Upper Cretaceous from Greenland to Alabama, while he considers that there is convincing evidence for the widespread occurrence of *Liriodendron* in the Cretaceous of North America. This view lends special interest to the above consideration of the stigmas in the Magnoliales.

Turning to another aspect of floral structure, it will be remembered that considerable reference has been made above to the carpels of the Rosaceae, and this at once suggests the criticism that the Rosaceae cannot be regarded as a primitive family because of their perigynous flowers. But what do we find in the early floras? The Leguminosae were undoubtedly present in the Eocene and probably in the Cretaceous, the Lauraceae were certainly well represented and there is fairly good evidence for the presence of other perigynous genera. These facts taken in conjunction with evidence derived from the Bennettitales and the Palaeozoic Pteridosperms (⁽²⁵⁾, p. 30) suggest that the hollow cup-like receptacle may be quite as primitive as the conical receptacle of the hypogynous flowers.

This paper does not pretend to be a final solution of the many morphological problems touched upon, but it is intended to open up new lines of thought and investigation. The author also hopes to provoke criticism, and to lead those who hold other opinions to examine and state the foundations of their beliefs.

SUMMARY

1. The whole structure of our current floral morphology rests on the assumption that the floral parts represent modified foliage leaves, and this view leads to further corollaries. But the fundamental concept has no trustworthy foundation, and there is little prospect of the discovery of new supporting evidence.

2. In attempting to build up a new system by considering modern floral structures in the light of what we know concerning the lines of evolution in reproductive structures generally, the approach must be objective and phylogenetic.

3. The basic assumptions underlying any scheme of morphology should be clearly stated, and consequently the postulates of the new morphology are set out.

4. A system of morphological concepts should be applicable to every structural feature both internal and external. The evidence

from nine different lines of enquiry has to be co-ordinated when we attempt to reach an explanation of carpel structure, and the explanation must be reasonable in relation to the physiology of the structures involved.

5. The morphological nature of the carpel, its origin and evolution are topics closely associated with the origin and evolution of the stigma.

6. If we assume that the carpel originated by the infolding of a fertile leaf serious difficulties arise when we try to envisage the possible modes of origin of the stigma and the physiological results of any process of infolding.

7. The study of the tissue along which the pollen tubes pass from the stigma to the ovules should throw light on the nature of the stigma. From the evidence now available the stigma appears to be a modified portion of the inner surface of the ovary wall, and the tissue along which the pollen tubes travel, here designated the transmitting tissue, becomes a feature of morphological importance.

8. Robert Brown's view that each simple carpel has two lateral stigmas is revived, and is illustrated by reference to the genus *Drimys*.

9. Juel's studies on carpel structure in the Rosaceae suggest ways in which a double lateral stigma arising near the base of the carpel may have become modified in the production of more efficient structures. Support to the suggestion is furnished by ontogenetic studies of diverse plants.

10. A new view of the possible origin of the stigma is brought forward which interprets the features previously noticed in modern plants by reference to the fossil Caytoniales and Pteridosperms. The carpel wall is regarded as derived from a pair of concrescent cupules, the two placentae representing fertile branch tips originally surrounded with a cupule. The stigmatic surfaces of a single carpel together with the transmitting tissue represent the marginal parts of the inner surface of the cupules lined with glandular hairs. The earliest stigmas arose near the lower part of the ventral side of the ovaries, but it is thought that where carpels or ovaries were crowded together natural selection would favour variation in which the stigma approached the free apex of the ovary.

11. This view agrees with what we know of the ontogeny of carpels; it explains the different forms of stigmas, especially the forked and commissural forms; it accounts for the main features of vascular anatomy, for the presence of transmitting tissue, and for the

prevalence of anatropous ovules. It may give a clue to the interpretation of the various types of abnormal carpels which have been described. The course of evolution suggested seems to be physiologically possible; it greatly simplifies the interpretation of the varied forms of carpels and ovaries.

12. Reference is made to a series of fossil plants belonging to successive geological epochs, which seem to show that the course of evolution in certain Pteridospermae was tending towards the structure postulated above. The Caytoniales are regarded as showing an actual transition from gymnospermy to angiospermy.

13. It is suggested that the chief sections of flowering plants may have arisen from different groups of pteridosperms, and that the characteristics of the earliest flowers may be ascertainable by studying the modern representatives of those genera or families which can be safely identified in the Upper Cretaceous and Eocene floras. The more primitive flowers may have been very different from the types hitherto postulated.

14. This contribution is intended to open up new lines of thought and investigation. It is a challenge to those who hold other views to reconsider and state the foundations of their opinions.

REFERENCES

- (1) ARBER, A. Floral anatomy and its morphological interpretation. *New Phyt.* **32**, 231. 1933.
- (2) ARBER, E. A. N. On some new species of *Lagenostoma*, etc. *Proc. Roy. Soc. B*, **76**, 245. 1905.
- (3) BERRY, E. W. Upper Cretaceous and Eocene floras of South Carolina and Georgia. *U.S. Geol. Survey, Prof. Paper* 84. 1914.
- (4) BHARGAVA, H. R. Contribution to the morphology of *Boerhaavia repanda*. *Journ. Indian Bot. Soc.* **11**, 303. 1932.
- (5) BROWN, R. On the relative position of the divisions of stigma, etc. (1840.) *Misc. Bot. Works of Robert Brown*, **1**, 555, Ray Soc. 1866.
- (6) CAPUS, G. Anatomie du tissu conducteur. *Ann. Sci. Nat. Bot.* **7**, 209. 1878.
- (7) FRIES, R. E. Zur Kenntniss der afrikanischen *Dorstenia* Arten. *Arkiv f. Bot.* **13**, 7. 1913.
- (8) GOEBEL, K. *Organography of Plants*. English ed. Oxford. 1905.
- (9) GRÉGOIRE, V. La valeur morphologique des carpels dans les Angiospermes. *Bull. Acad. Roy. Belgique, Cl. Sci. Sér. 5*, **17**, 1286. 1931.
- (10) GUÉGUEN, F. Recherches sur le tissu collecteur et conducteur des Phanérogames. *Journ. Bot.* **14**, 140, 165. 1900.
- (11) ——— Anatomie comparée du tissu conducteur du style et du stigmat des Phanérogames. *Journ. Bot.* **15**, 265. 1901; **16**, 15, 48, 138, 167, 280, 300. 1902.
- (12) HARRIS, T. M. A new member of the Caytoniales. *New Phyt.* **32**, 97. 1933.
- (13) JOSHI, A. C. and RAO, V. S. Floral anatomy of *Rivina humilis* L., etc. *New Phyt.* **32**, 359. 1933.

- (14) JUEL, H. O. Beiträge zur Blütenanatomie und zur Systematik der Rosaceen. *K. Svensk Vet. Akad. Hand.* **58**, No. 5. 1918.
- (15) JULIANO, J. B. Floral morphology of *Lyonothamnus floribundus*. *Bot. Gaz.* **91**, 426. 1931.
- (16) LAMPRECHT, H. Ein unifoliata-Typus von *Pisum* mit gleichzeitiger pistilloidie. *Hereditas*, **18**, 57. 1933.
- (17) MAHESHWARI, P. Contributions to the morphology of *Boerhaavia diffusa*. *Journ. Indian Bot. Soc.* **8**, 219. 1929.
- (18) MURBECK, S. Ueber das Verhalten des Pollenschlauches bei *Alchemilla arvensis*, etc. *Lunds Univ. Årsskrift*, **38**, 3. 1901.
- (19) OLIVER, F. W. and SALISBURY, E. J. Structure and affinities of the Palaeozoic seeds of the Conostoma group. *Ann. Bot.* **25**, 1. 1911.
- (20) PAYER, J. B. *Traité d'organogénie comparée de la fleur*. Paris. 1857.
- (21) SALISBURY, E. J. On the morphology and ecology of *Ranunculus parviflorus* L. *Ann. Bot.* **45**, 539. 1931.
- (22) SCHNARF, K. Embryologie der Angiospermen. *Handbuch der Pflanzen-anatomie*, Abt. 2, Teil 2. Berlin. 1929.
- (23) THOMAS, H. HAMSHAW. The Caytoniales, etc. *Phil. Trans. Roy. Soc. B*, **213**, 299. 1925.
- (24) — The early evolution of the Angiosperms. *Ann. Bot.* **45**, 647. 1931.
- (25) — The old morphology and the new. *Proc. Linn. Soc.* **145** (1932–3), 17. 1933.
- (26) — On some pteridospermous plants from the Mesozoic rocks of South Africa. *Phil. Trans. Roy. Soc. B*, **222**, 193. 1933.
- (27) THOMPSON, J. M. The theory of the Leguminous strobilus. *Proc. Linn. Soc.* **144** (1931–2), 98. 1932.
- (28) — The theory of Scitamincan flowering. *Pub. Hartley Bot. Lab. Liverpool*, **11**. 1933.
- (29) TROLL, W. Morphologie der schildförmigen Blätter. Teil II. *Planta*, **17**, 231. 1932.

THE QUANTITATIVE DETERMINATION OF MINUTE AMOUNTS OF CHLOROPHYLL

By PHYLLIS A. HICKS, M.Sc., Ph.D. AND

THÉRÈSE E. PANISSET, B.Sc., A.R.C.S.

Research Institute of Plant Physiology, Imperial College of
Science and Technology, London

(With 5 figures in the text)

INTRODUCTION

IN the course of an experiment upon the growth of *Lemna* under different light intensities the most obvious difference between the cultures was found to be that of colour. The low light cultures were richly green, those grown under strong light were of a pale yellowish colour, while those grown under light of intermediate intensities exhibited intermediate shades of green. It was felt therefore that some method of estimating the chlorophyll content should be valuable if the interpretation of the effect of the light intensity factor was to be considered.

One of the standard methods of estimation of this pigment is that of the spectrophotometer. It was exploited primarily by Monteverde⁽¹¹⁾ in 1893 and extended by Malarski and Marchlewski⁽⁹⁾, who converted their chlorophyll into chlorophyllan and measured the absorption of this solution by a König-Martens spectrophotometer. Wurmser and Declaux⁽¹⁹⁾ applied the spectrophotometer method to the determination of the pigments in the red algae, but did not suggest a fixed basis for comparison as did Lubimenko⁽⁷⁾, who, working on similar material, used crystallised chlorophyll prepared by the method of Willstätter and Stoll⁽¹⁸⁾. The above methods were only applicable where large quantities of pigment could be extracted, but Jacobson⁽⁵⁾ refined the method for the determination of the amount of the two chlorophylls present in 1 grm. or less of the material. Later Dastur and Buhariwalla⁽¹⁾, using a quartz ultra-violet spectrograph with a photographic attachment, determined small quantities of chlorophyll from tropical plants, by means of which they compared the chlorophyll content of successive leaves, but their method entailed the initial use of 500 grm. of leaf powder to prepare a standard solution.

Jacobson's method was tried but after much experimentation it was found impossible to make comparable chlorophyllan extracts from such small quantities as 1 mg. of dried tissue, which was the maximum weight of material available for use in the case of *Lemna*. Any process entailing a conversion from the original extract produced an experimental error much too large for practical purposes.

A much more readily available method of pigment determination is based upon the use of the colorimeter. The spectro-colorimeter was suggested in 1913 by Monteverde and Lubimenko(12), and in 1919 Henrici(3), using an alcoholic extract of crude chlorophyll, made colorimetric comparison of chlorophyll concentration; unfortunately, however, the results were not placed upon an absolute basis. Four years later Maiwald(8), by means of a Dubosc colorimeter and a standard of pure chlorophyll ($a + b$) procured from Willstätter, obtained satisfactory comparisons.

In recent years Schertz(13) has carefully reviewed and tested the above methods. He was doubtful whether any of the writers referred to had used really pure chlorophyll, so he prepared and purified a sample according to the instructions of Willstätter and Stoll(18). When this was done, a known amount, i.e. 0.0507 gm., was weighed out and dissolved in ether. The solution was then saponified by shaking with 10 c.c. of cold methyl alcoholic potash for 15 minutes, the ether evaporated and the solution of potassium chlorophyllin made up to 250 c.c., from which standard dilutions could be prepared. Schertz used Lovibond colour slips to estimate the depth of colour rather than the tint, and the depth was measured on a Dubosc colorimeter. This method, accurate as it may be for larger quantities, could not be used for small amounts for the reasons previously stated, but its value in connection with this experiment lies in the facts that Lovibond slides were used, and that in comparing this method with that of the spectroscope, Schertz found that results could more easily be obtained by the colorimeter method and with the same degree of accuracy.

EXPERIMENTAL METHODS

The crude alcoholic extracts from ten fronds of *Lemna minor* in 0.2 c.c. alcohol could be analysed by colorimetric methods using Lovibond slips. The values for blue ranged from 2 to 3.5, while yellow remained fairly constant at 11.5. It was hoped that with this extract a method might be devised to meet microchemical needs.

First a fixed standard of pure chlorophyll was required. The

Determination of Minute Amounts of Chlorophyll 201

market was explored for samples of this. Two samples of a liquid technical product were obtained, but all attempts to purify and crystallise this failed, presumably due to copper chlorophyllide contamination. A pure product could not be obtained commercially. Next an attempt was made to prepare chlorophyll from nettle leaves and *Lemna* plants. The method of Schertz was used but it was found impossible, even after frequent purifications, to obtain dry crystals; a sticky, inconstant product was always produced. On consultation with Dr Schertz it was learned that sticky chlorophyll was usually the result, but that a later method, not yet published, resulted in good dry crystals. A gram of these was very kindly sent to us and after tests of purity was used as a basis for comparison.

Purity tests (Jørgensen and Kidd⁽⁸⁾). Chlorophyll is *saponified* by strong alkalis to form water-soluble chlorophyllins, the yellow pigments not being attacked. A 30 per cent. solution of potassium hydroxide is added to an ethereal solution of chlorophyll and, after saponification is complete, water is added. Green chlorophyllin salts separate out in the aqueous layer and any yellow pigments are detached in the ethereal layer. Using this test, we found that the upper layer remained perfectly colourless, showing the chlorophyll to be free from yellow pigments.

Allomerisation. Unaltered and pure chlorophyll on saponification with alkali gives a brown colour, which after a few minutes changes back to green. Allomerised chlorophyll does not give this brown phase on saponification. We found that the brown phase was exhibited by Schertz's sample.

Ash weight. The ash weight of pure chlorophyll should be 4.5 per cent. of the dry weight, and consist of pure MgO. In a test, the weight of chlorophyll (Schertz) was 2.230 gm., which gave an ash weight of 0.101 gm.; thus the ash weight per percentage dry weight was 4.5. The ash was pure white and gave no flame tests.

Having ascertained the purity of the chlorophyll, a suitable solvent for both the prepared pigment and plant plastids was sought.

Solubility of chlorophyll. (1) Methyl alcohol (80 per cent.). Pure dry chlorophyll is only sparingly soluble in cold methyl alcohol but will go into solution if it is powdered and the alcohol warmed. Plant tissues previously killed in boiling water have their pigments readily extracted by warm alcohol, giving a good green solution. (2) Ether. Prepared chlorophyll dissolves with great ease and rapidity in cold ether, giving a very deep blue-green solution, but it is difficult to extract the pigments from plant tissues with this solvent even if the

tissues are first killed by boiling, or by drying, a process not recommended. (3) Acetone. Chlorophyll is readily soluble in this reagent even in the cold, but again acetone is not a very good solvent for plant pigments. Killed fresh material is not readily affected by it, while dried material gives but a yellowish solution.

Methyl alcohol (80 per cent.) was thus adopted as a solvent. About 3 mg. of finely powdered chlorophyll were weighed out into a platinum

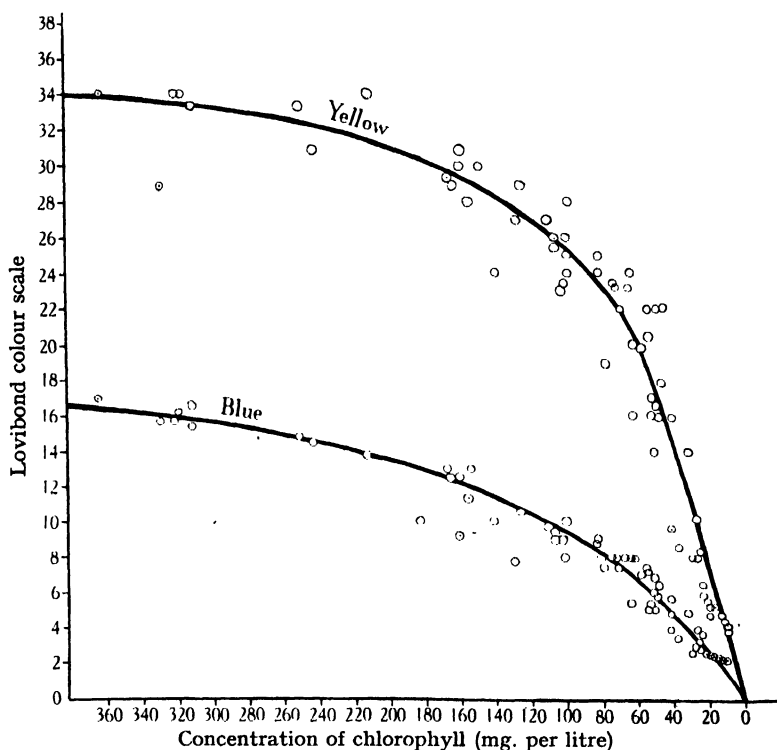


Fig. 1. Curve showing the colour values obtained with different concentrations of chlorophyll dissolved in 80 per cent. methyl alcohol.

boat, dissolved in warm alcohol and the solution made up to 10 c.c. Dilutions were made immediately afterwards and the stock solution kept in the dark. It was subsequently found that chlorophyll solutions decomposed even in the dark within a few days, as was shown by a distinct lowering of the value for the blue colour. Dilution curves (Fig. 1) were obtained.

A crude alcoholic extract of green plant tissue contains flavones (colourless or yellow), carotin and xanthophyll (yellow to orange),

Determination of Minute Amounts of Chlorophyll 203

and chlorophyll *a* and *b* (green). It will be observed that all of these pigments contain the yellow colour factor; the chlorophylls are unique in possessing a blue factor, and the carotin and xanthophyll group a red factor. Does this blue factor remain constant when additions of red and yellow pigments are made to it? To determine this, solutions of carotin were added to pure chlorophyll solutions. The carotin, a crystalline powder, was kindly supplied by Mr A.

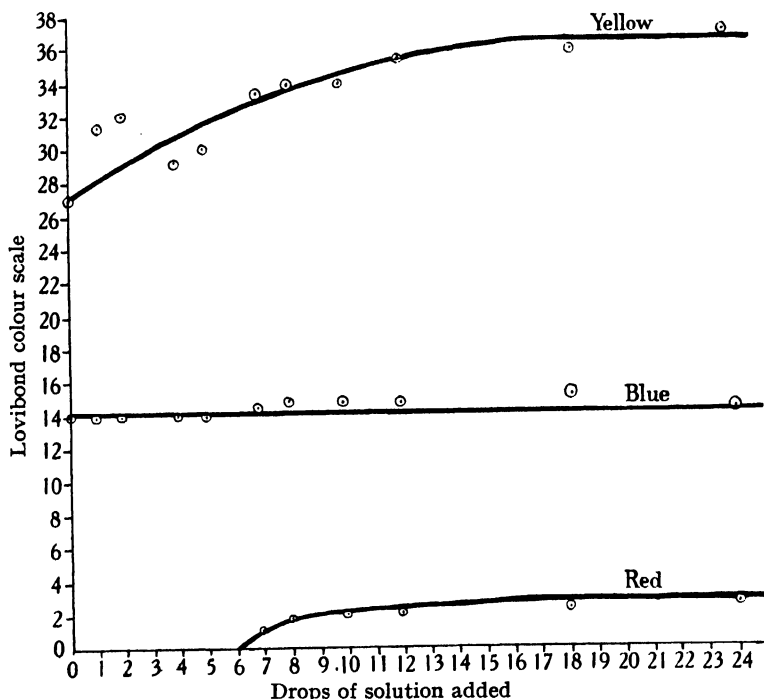


Fig. 2. Curve showing the colour values obtained from a solution of chlorophyll to which carotin has been added in increasing concentration.

Pollard of this College. In order to make comparable dilutions with very small quantities of the pigment a solution was prepared by adding a very little solid carotin to a solution of chlorophyll of known strength, and this mixed solution was added gradually in micro-drops to the chlorophyll solution. This method prevented colour differences due to a gradual dilution of the original solution which would have occurred if an alcoholic solution of carotin had been added. A curve was obtained (Fig. 2) which shows that the blue colour remains constant.

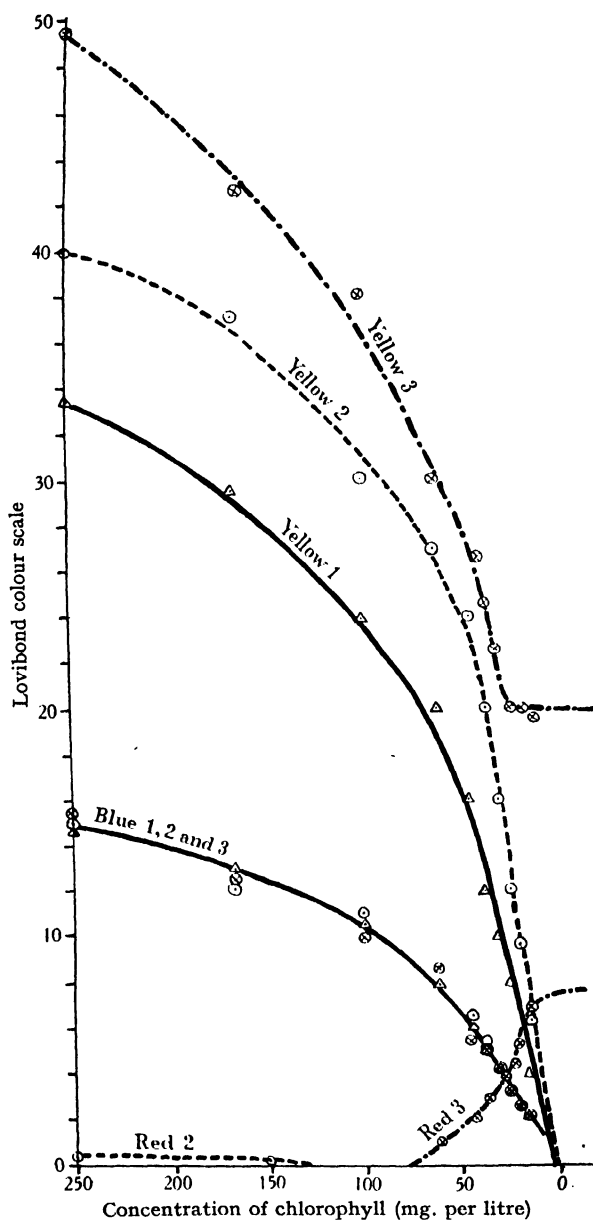


Fig. 3. Curve showing the colour values obtained when a solution containing chlorophyll and carotin is diluted. Blue 1 and Yellow 1 = values for pure chlorophyll. Blue 2, Yellow 2 and Red 2 = values for chlorophyll plus carotin (both diluted equally). Blue 3, Yellow 3 and Red 3 = values for chlorophyll plus carotin (concentration of carotin increasing, of chlorophyll decreasing).

Determination of Minute Amounts of Chlorophyll 205

A further test was made by adding a little carotin to a concentrated solution of chlorophyll of known strength and diluting the mixture with known proportions of alcohol. The graph given in Fig. 3 (Blue 2, Yellow 2, Red 2) shows that the blue colour values lie along the curve calculated for chlorophyll alone. A third check was made by adding to each of a gradually diluted series of chlorophyll concentrations a fixed amount, i.e. one micro-drop, of a concentrated solution of carotin. This gave a series of increasing carotin concentrations against decreasing chlorophyll concentrations, and the results, also shown in Fig. 3 (Blue 3, Yellow 3, Red 3), demonstrate that the blue colour remains unchanged.

In all these colour matchings it is important to notice that the *depth* of colour must be taken into account as well as the actual *tint*. If the tint only is matched errors will be obtained due to an absorption of the blue light by the added yellow pigments; this is allowed for if the *depth* of colour is taken into account. A little practice with the colorimeter, and solutions which are not so concentrated as to appear muddy in the comparator, will soon make the exact matching of colour and depth quite simple.

A fixed standard of pure chlorophyll in 80 per cent. alcohol being available, the alcoholic extract of pigment from the plant is placed in the comparator, the colour matched in red, yellow and blue, and the blue value sought upon the curve for pure chlorophyll. This gives the *absolute* chlorophyll concentration; the red colour similarly gives the *relative* proportion of carotin and xanthophyll present.

THE COLORIMETER

The apparatus used in this experiment is depicted in Fig. 4. One advantage of the method employed is that very little special apparatus is required. The comparator can easily be made with cardboard painted with brunswick black. It consists of a box about 4 in. square with a piece of daylight glass (*A*) in the top. The front consists of a sheet of ordinary white glass (*B*), while a sheet of opal glass (*C*) is placed diagonally inside the box so as to reflect through the chlorophyll solutions the light of a 100 watt lamp situated above the daylight glass. The tapering eyepiece (*D*) can also be made of blackened cardboard; it should contain a small hole for the eyepiece (*E*) and slots (*S*) to hold the Lovibond slides. An improvement is a small system of mirrors placed between the eyepiece and the colour slips to reflect the colours so that they appear to lie side by side; this makes comparison easier. Two large slots (*F*) hold the vessels containing the

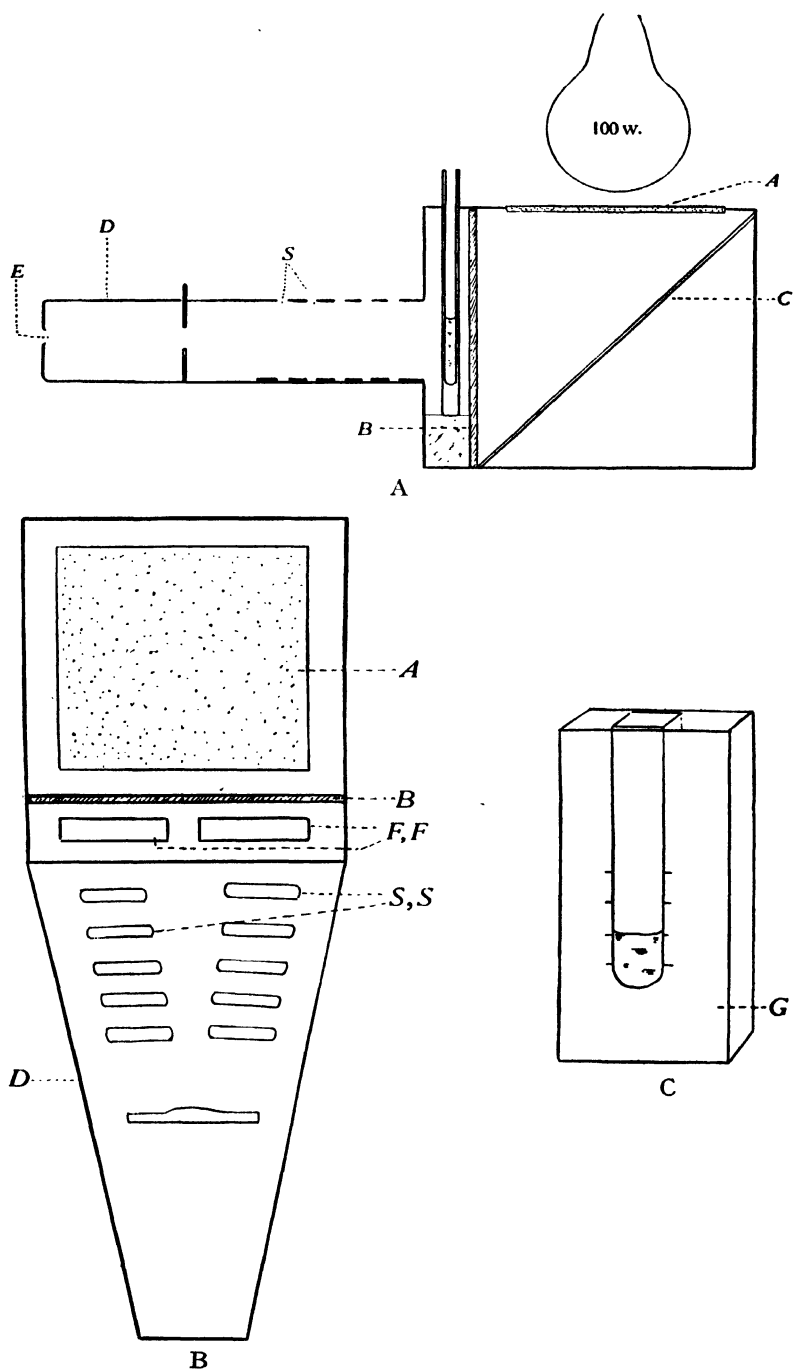


Fig. 4. Diagram of apparatus used. Explanation in text. A, section; B, top view; C, vessel for solution.

Determination of Minute Amounts of Chlorophyll 207

solutions; chlorophyll solution is placed in one vessel, while, in order to make conditions of matching as alike as possible, a similar container with distilled water is placed in the other slot. Pieces of colourless glass are placed in the slots in front of the chlorophyll solution as a control for the Lovibond slides in front of the vessel of water. The special flat-sided container (G), with calibration mark, has an advantage over a cylindrical one in the fixed depth of solution for absorbing light.

The same colorimeter, illuminating lamp, and vessel in which the chlorophyll solution is placed must be used throughout any one experiment. Any change in the apparatus must be followed by a fresh calibration of the chlorophyll as the colour values are not absolute but relative to the amount of light received and absorbed by the solution.

Since chlorophyll is difficult to prepare and impossible to purchase in the open market, it might be advantageous to evaluate each colour standard of the chlorophyll concentrations in the original calibration with a solution of some stable compound such as aqueous malachite green, as suggested by Sprague and Shive(15). This would save the use of pure chlorophyll in any fresh calibration.

THE APPLICATION OF CHLOROPHYLL DETERMINATIONS

Many investigators have drawn attention to the need for a simple determination of chlorophyll, since chlorophyll content is related to plant metabolism, chemical composition and area, and is influenced by such factors as light intensity and manurial treatment. Willstätter and Stoll(18) found a relationship between chlorophyll content and the rate of photosynthesis, while an experiment carried out on *Lemna minor* along lines similar to their work established a relationship between light intensity, chlorophyll content and respiration rate(4). See also Table I below and Fig. 5.

A correlation between nitrogen content and leaf colour was established by Ville(17) as early as 1889 and corroborated by Urban(16) in 1918, while Maiwald(8) and Schertz(14) both show that differences in chlorophyll content are caused by potassium. These facts, taken with the findings of Gregory and Richards(2) on the striking effects of these two mineral nutrients upon the respiration and assimilation rates of barley leaves, seem to indicate a simple relationship between mineral nutrition, chlorophyll content and the rate of metabolic exchange.

A chlorophyll/protein relationship was inferred by Meyer(10), a fact which Sprague and Shive used as a basis to explain the relation-

ships between chlorophyll content, total dry weight and leaf area which they found in their investigations.

More data along these lines are obviously required, and with a

TABLE I

Milligrams of CO₂ respired per hour by frond material containing 1 mg. chlorophyll; 25° C.

Days	Foot-candles			
	1400	1000	750	350
0	0.70	0.70	0.70	0.70
5	0.88	1.16	0.67	1.08
9	0.96	1.10	1.05	0.96
16	0.81	0.88	1.10	1.07

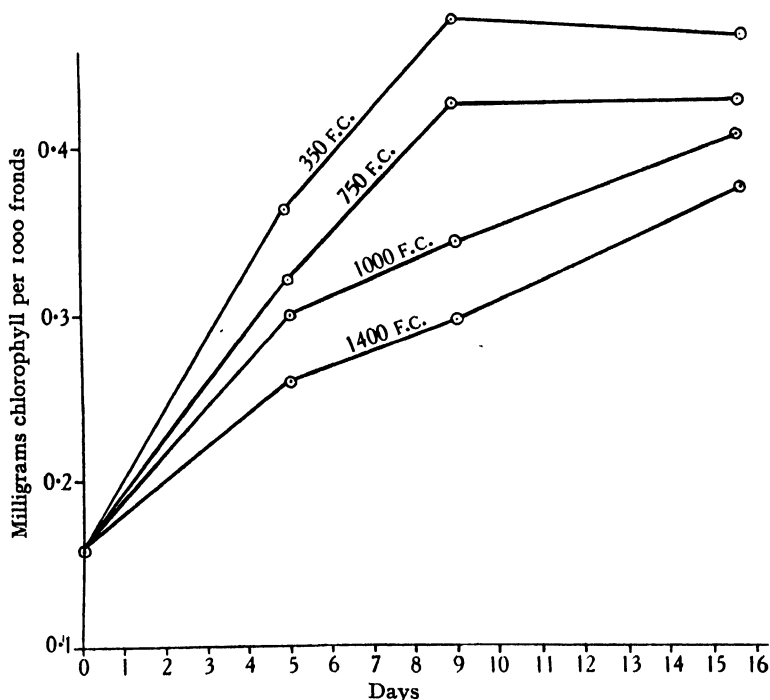


Fig. 5. Graph of chlorophyll content of *Lemna* fronds grown under four different light intensities.

method of chlorophyll determination such as has been described above the relationships could be worked out leaf by leaf so quickly that the large number of possible estimations would minimise both sampling and experimental error.

Determination of Minute Amounts of Chlorophyll 209

It may also be pointed out that the above method, although primarily for small quantities of material, is equally useful for diluted extracts from larger masses of material.

We are indebted to Dr F. M. Schertz of the United States Department of Agriculture, who kindly provided a sufficient quantity of pure crystalline chlorophyll to enable us to make a chlorophyll standard, and to Mr A. Pollard of the Imperial College of Science and Technology for the gift of carotin.

The work was undertaken at the Imperial College of Science and Technology under the supervision of Prof. V. H. Blackman and Dr F. G. Gregory, whose help on many points was gratefully appreciated.

SUMMARY

An apparatus is devised whereby accurate estimation may be made of the amount of chlorophyll ($a+b$) in the crude extract (in 80 per cent. methyl alcohol) from 5 to 20 mg. of fresh material. The method is a colorimetric one and is based on the constancy of absorption in the blue, when the depth as well as the colour is matched with Lovibond colour slides in a specially designed colorimeter.

Data are given of the chlorophyll content of *Lemna* fronds grown under four different intensities of light, and the possibility of a simple relationship existing between respiration rate and chlorophyll content is indicated.

Various applications of the method described are suggested.

REFERENCES

- (1) DASTUR, R. H. and BUHARIWALLA, N. A. Chlorophyll from tropical plants and its quantitative determination by means of the spectrograph. *Ann. Bot.* **42**, 949-64. 1928.
- (2) GREGORY, F. G. and RICHARDS, F. J. Physiological studies in plant nutrition. I. The effect of manurial deficiency on the respiration and assimilation rate in barley. *Ann. Bot.* **43**, 119-61. 1929.
- (3) HENRICI, M. Chlorophyllgehalt und Kohlensäure-assimilation bei Alpen- und Ebenenpflanzen. *Verhandl. naturf. Ges. Basel*, **30**, 43-136. 1919.
- (4) HICKS, P. A. Some preliminary observations upon the interaction of light and temperature on the growth of *Lemna*. *Ann. Bot.* **48**, 515-25. 1934.
- (5) JACOBSON, C. A. A delicate method for determining minute quantities of chlorophyll. *Journ. Amer. Chem. Soc.* **34**, 1266-8. 1912.
- (6) JÖRGENSEN, I. and KIDD, F. Some photochemical experiments with pure chlorophyll and their bearing on the theories of carbon assimilation. *Proc. Roy. Soc. B*, **89**, 1. 1917.
- (7) LUBIMENKO, V. Sur la quantité de la chlorophylle chez les algues marines. *Compt. Rend. Acad. Sci. Paris*, **179**, 1073-6. 1924.

210 PHYLLIS A. HICKS AND THÉRÈSE E. PANISSET

- (8) MAIWALD, K. Wirkung hoher Nährstoffgaben auf den Assimilationsapparat. *Angew. Bot.* **5**, 33-74. 1923.
- (9) MALARSKI, M. and MARCHLEWSKI, L. Studien in der Chlorophyllgruppe. VI. Bestimmung des Chlorophylls in Pflanzenteilen. *Biochem. Zeits.* **24**, 319-22. 1910.
- (10) MEYER, A. Eiweisstoffwechsel und Vergilben der Laubblätter von *Tropaeolum majus*. *Flora*, **111**, 85-127. 1918.
- (11) MONTEVERDE, N. A. Das Absorptionsspectrum des Chlorophylls. *Acta Horti Petropolitani*, **13**, 123-78. 1893.
- (12) MONTEVERDE, N. A. and LUBIMENKO, V. Formation of chlorophyll in plants. III. Application of the spectro-colorimetric method of quantitative analysis to the study of accumulation of chlorophyll, xanthophyll and carotin in the plant. *Bull. Acad. Sci. St Petersburg*, **7**, 1007-28. 1913.
- (13) SCHERTZ, F. M. The quantitative determination of chlorophyll. *Pl. Phys.* **3**, 323-34. 1928.
- (14) — The effect of potassium, nitrogen and phosphorus fertilising upon the chloroplast pigments, upon the mineral content of the leaves and upon the production in crop plants. *Pl. Phys.* **4**, 269-79. 1929.
- (15) SPRAGUE, H. B. and SHIVE, J. W. A study of the relations between chloroplast pigments and dry weights of tops in dent corn. *Pl. Phys.* **4**, 2. 1929.
- (16) URBAN, J. Über die Farbe des Rübenkrautes früh- und spätreifender Rüben. *Zeits. Zuckerind. Böhmen*, **42**, 281-97. 1918.
- (17) VILLE, G. Recherches sur les relations qui existent entre la couleur des plantes et la richesse des terres en agents de fertilité. *Compt. Rend. Acad. Sci. Paris*, **109**, 397-400. 1889.
- (18) WILLSTÄTTER, R. and STOLL, A. *Untersuchung über Chlorophyll*. Berlin. 1913.
- (19) WURMSER, R. and DECLAUX, J. Sur la photosynthèse chez les algues floridées. *Compt. Rend. Acad. Sci. Paris*, **171**, 1231-3. 1921.

THE ROOTING OF CUTTINGS OF *LONICERA JAPONICA*: A PRELIMINARY ACCOUNT

By SAMUEL SANDISON, B.Sc.

From the Department of Botany, University of St Andrews,
University College, Dundee

(With 5 figures in the text)

IT was found in *Lonicera japonica* that adventitious roots occur frequently in more or less definite regions in the normal growing shoot, and are visible externally as slight swellings in the bark. These roots are generally to be found at the node, in a median or lateral position just below the insertion of a leaf, but are, however, to be found to a lesser degree at any part of the internode. They occur most frequently at nodes where there has been a strong growth of the axillary shoots. Hence they are commonest in the lower regions of the plant.

The first cuttings examined were taken from a stunted and pot-bound plant, and showed numerous root primordia. It was found that other, more vigorous, plants showed fewer root primordia. In connection with this phenomenon, wild specimens of *Lonicera periclymenum* were examined, and visible root primordia were seen, while on a cultivated specimen of the same plant no root primordia were visible. Further work is to be done to determine whether there is any definite correlation between nutrition and the development of root primordia in *Lonicera* spp.

In propagated cuttings, roots were found to arise from any part of the stem, those cuttings which were taken just below the node being most successful. The number of roots developed on the cuttings was such as led one to believe that they were not all produced from pre-existing root initials.

Upon investigation it was found that there were striking differences in the type of root produced, dependent upon the point and mode of origin, namely:

- (a) Roots developed from pre-existing root initials.
- (b) Roots induced as a result of vegetative propagation.

The external appearances of these two kinds of roots are shown in Fig. 1.

ROOTS FROM PRE-EXISTING ROOT INITIALS

Appearance. These roots are relatively thick, unbranched, white in colour and soft in texture (Fig. 1a).

Origin. They are generally to be found proceeding from the median and lateral leaf-trace gaps below the insertion of a leaf (Fig. 1a), but

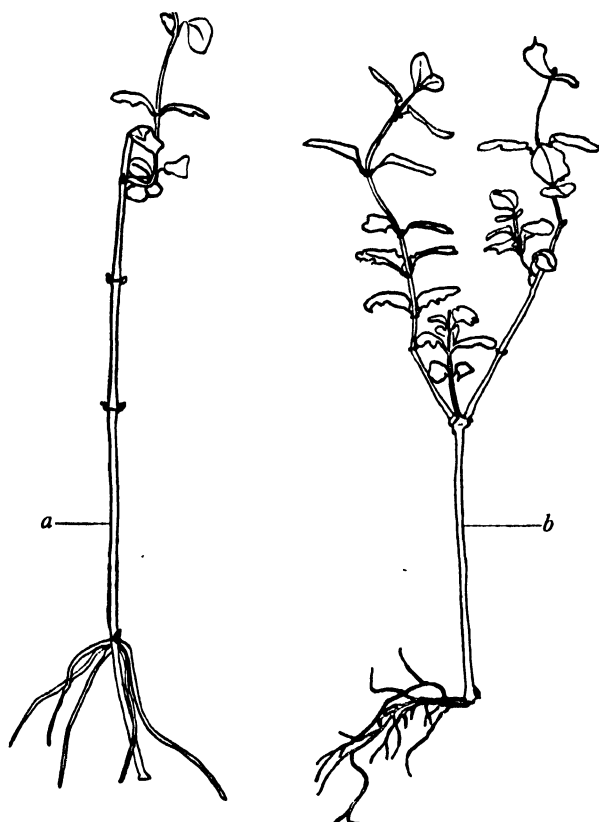


Fig. 1. Left-hand cutting (a) with preformed roots. Right-hand cutting (b) with induced roots. Drawing from photograph. $\times \frac{1}{2}$.

All the sections illustrated were cut by sliding microtome without embedding, about 15μ thick, and stained with Safranin and Delafield's Haematoxylin. Photograph on Eastman Commercial Ortho Film, Wratten B screen, Watson's $\frac{3}{8}$ in. objective, no eyepiece.

this is not always the case. They are to be found at any part of the internode.

Development. The earliest stages in development of such a root would seem to show that a group of cells extending from the cambium to the pericycle has remained, or again become, meristematic, and has

become reorganised as a root. The greatest degree of organisation occurs in the pericycle, and, from signs of displacement in the cells of the xylem surrounding the base of the root, it is supposed that organisation has proceeded backwards from the pericycle (Fig. 2). Differentiation of wood elements occurs only at a late stage, and towards the base of the root, and is not very pronounced. The medulla of the root is occupied by parenchymatous cells. In this condition of slow differentiation and slow growth the root remains visible only as a swelling in the bark, which it may eventually rupture.

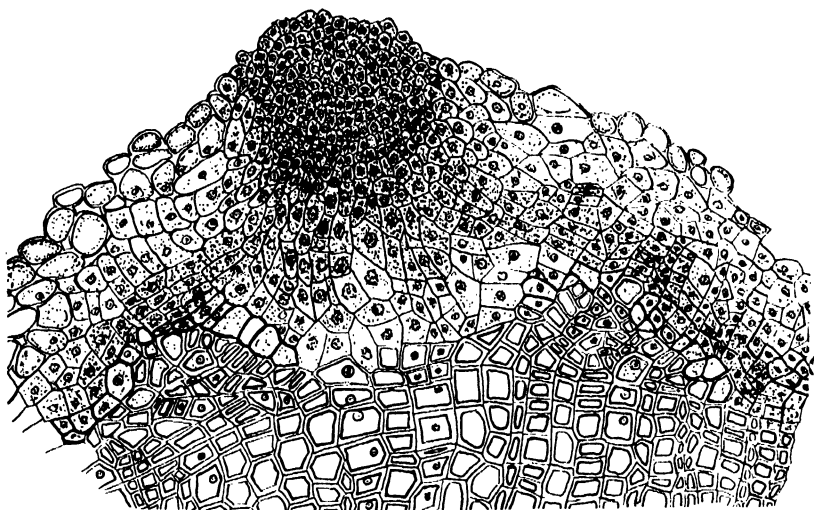


Fig. 2. Base of preformed root initial, showing displacement of cells of xylem of the stem surrounding the base of the root. Camera lucida. $\times 300$.

Anatomy. As shown by a transverse section, this type of root is characterised by the small amount of wood formed. The stele is medullated, and the strands of xylem, which may be from two to five in number, are only one or two cells in width. There is a fairly wide cortex, and a well-marked pericycle and endodermis (Fig. 3).

ROOTS INDUCED BY VEGETATIVE PROPAGATION

Appearance. These roots are relatively thin, extensively branched, brownish in colour and fibrous in texture (Fig. 1*b*).

Origin. They are only to be found at the end of a cutting, usually in conjunction with callus tissue (Fig. 1*b*). In one case a root of this type was seen to be developing a short distance up the stem, but here

also its appearance coincided with the production of callus following upon the removal of a bud.

Development. It was found that propagation of a cutting caused the renewal of cambial activity at the basal end (see also Sledge(3)).

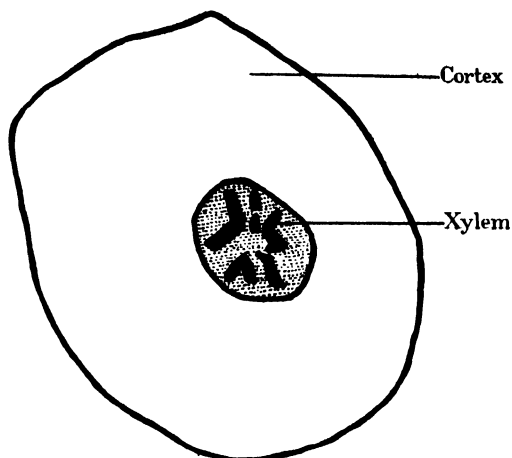


Fig. 3. Transverse section of young root developed from preformed root initial, to show lignification of stele. Semi-diagrammatic drawing from photomicrograph. $\times 65$.

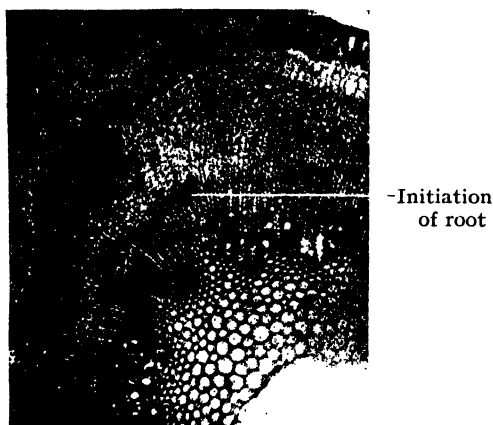


Fig. 4. Transverse section of stem just above callus surface, showing group of cambial cells reorganising as a root apex. $\times 25$.

This renewal of cambial activity is associated with the production of callus, and also the formation of cells to the inside of the cambium facing the stele, which later lignify. In addition a group of cambium cells may reorganise as a root apex (Fig. 4). Growth in length pro-

ceeds quickly; a solid core of meristematic tissue extending from the root apex to the differentiating vascular elements of the stele characterises such a root. Differentiation in the root proceeds immediately, and leads to the formation of a solid core of xylem elements, spirally thickened, which are applied to the lignified elements of the stem.

At the same time the differentiating root is surrounded by a meristematic zone of cells, continuous with the cambium, which is adding to its growth. The formation of peculiar nests of wound wood, developed from the cambium, and surrounded by a sheath of meriste-

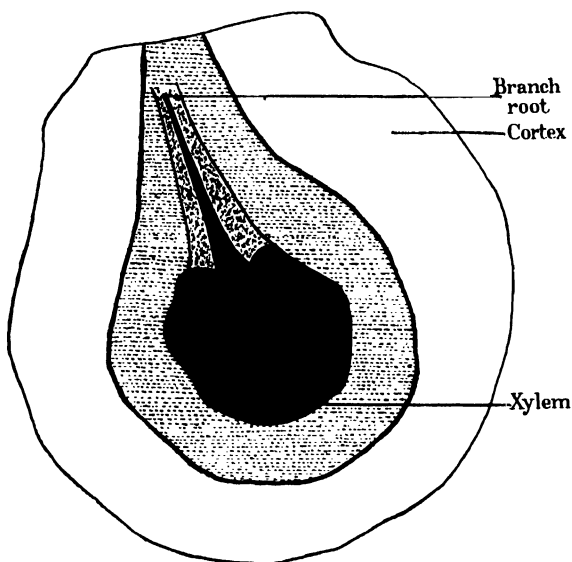


Fig. 5. Transverse section of young root induced by propagation, to show lignification of the stele. Semi-diagrammatic drawing from photomicrograph. $\times 65$.

matic cells, resembles so closely the development of a root that there seems little doubt, in this case at least, that the two processes are very closely related.

Anatomy. In contrast with the root developed from a pre-existing root initial, the induced root is characterised by a stele with a massive core of xylem (Fig. 5).

Two kinds of roots have been discovered by White(5) to co-exist as part of the normal morphology of the strawberry, and here also the anatomical features associated with the branching type of root lie in the heavy lignification of the stele, and the thin fibrous appearance.

The non-branching root is relatively thicker, whiter and softer, and shows a small amount of lignification.

Brenchley and Jackson⁽¹⁾ mention similar roots found in certain monocotyledons, while Cocks⁽²⁾ describes the transition of a non-branching root of *Camassia* to a branching condition on transference to dry fibre. In the case of *Lonicera japonica* the non-branching type of root was induced to branch by transference to water. The branch roots here were of the medullated type, similar to the roots from which they sprang.

Van der Lek⁽⁴⁾ mentions two kinds of roots which he describes as being discernible on close observation:

(a) "Roots that develop in the immediate neighbourhood of the basal wound surface."

(b) "Roots that in their appearance are bound to certain places morphologically determined."

The former he indicates as *wound roots* and the latter as *morphological roots*.

As far as can be gathered, the difference between these two types of roots is merely a positional one; but while Van der Lek's definition of the roots agrees very closely with the roots obtained from cuttings of *Lonicera japonica*, the latter, as has been shown, differed in appearance, origin, and structure. It is felt that a closer study of the material used by Van der Lek, and indeed of all types of cuttings, will reveal more than a mere positional difference between wound roots and morphological roots.

The writer wishes to express his thanks to Dr E. Philip Smith for her advice and guidance during this investigation.

SUMMARY

1. Visible root initials are found to be more numerous on starved and stunted plants of *Lonicera japonica* than on more healthy specimens.

2. When cuttings are propagated, two kinds of roots are obtained. These roots differ in appearance, in origin, and in morphology.

3. One type is developed from preformed root initials. The second type of root is formed from the cambium, and is one of the expressions of the latter's response to the wounding and propagation of the stem. They are identified with the wound roots and the morphological roots described by Van der Lek.

REFERENCES

- (1) BRECHLEY, W. E. and JACKSON, V. G. Root development in barley and wheat under different conditions of growth. *Ann. Bot.* **35**, 533-56. 1921.
- (2) COCKS, A. M. The growth habit of the monocotyledonous root system. Unpublished Thesis. Leeds. 1925. (Reference from Priestley and Swingle.)
- (3) SLEDGE, W. A. The rooting of woody cuttings. *Journ. of Pomology and Horticultural Science*, **8**, reprint, p. 5. 1930.
- (4) VAN DER LEK, H. A. A. Root development in woody cuttings. *Landbouwhoogeschool Wageningen, Verhand.* **1**, 219. 1924.
- (5) WHITE, P. R. Studies of the physiological anatomy of the strawberry. *Journ. Agr. Res.* **35**, 481-92. 1927.

THE CORRELATION OF MORPHOLOGICAL VARIATION WITH DISTRIBUTION IN SOME SPECIES OF *AJUGA*

By W. B. TURRILL, D.Sc., F.L.S.

(With 15 figures and 1 map in the text)

THE genus *Ajuga*, as at present usually understood, consists of about ninety species, mostly distributed in the North Temperate Zone of the Old World. The occurrence of the genus in South Africa and still more in Australia (see *Bot. Mag.* t. 9320) opens up very interesting phytogeographical problems which, however, it is not the purpose of this paper to discuss. The species with whose distribution we are here concerned are limited to Europe and the Nearer East and are morphologically quite distinct from other species of the genus. Fortunately their specific nomenclature is clear and it is not necessary to give a complete synonymy.

Ajuga Chamaepitys Schreb. *Unilab.* XXIII (1774) was first named *Teucrium Chamaepitys* by Linnaeus, *Sp. Pl.* 562 (1753), and the species must by the International Rules date from this latter publication. Linnaeus gives the "Habitat" as "in Italiae, Galliae, Angliae, Hungariae, Helvetiae arvis."

Ajuga chia Schreb. *l.c.* XXV (1774) is based on Tournefort's "*Chamaepitys chia*, folio trifido, magno flore" from the island of Chios. It is acknowledged to be very similar to *A. Chamaepitys*, but to differ in being more slender and having larger flowers which exceed the subtending leaf in length and have deeper lip crenations.

Two varieties of *A. Chamaepitys* have been described by Visiani. The var. *grandiflora* Vis. *Flor. Dalm.* 222 (1847), from near Sebenico, has the flowers nearly equalling the subtending leaves. The var. *glabra* Vis. *Flor. Dalm. Suppl.* 91 (1872), from about Stobreč, near Spalato, has puberulous stems and leaves and the calyces very glabrous. The latter plant was originally described as a species by Presl, *Flor. Sic.* i, XXXVI (1826). Apparently the same plant was described by Holuby from Hungary as var. *glabriuscula* in *Oester. Bot. Zeits.* XXII, 79 (1872), and this name probably has slight priority over Visiani's use of "*glabra*" as a varietal epithet.

Freyn in *Oester. Bot. Zeits.* XXVI, 405 (1876) discusses the value of

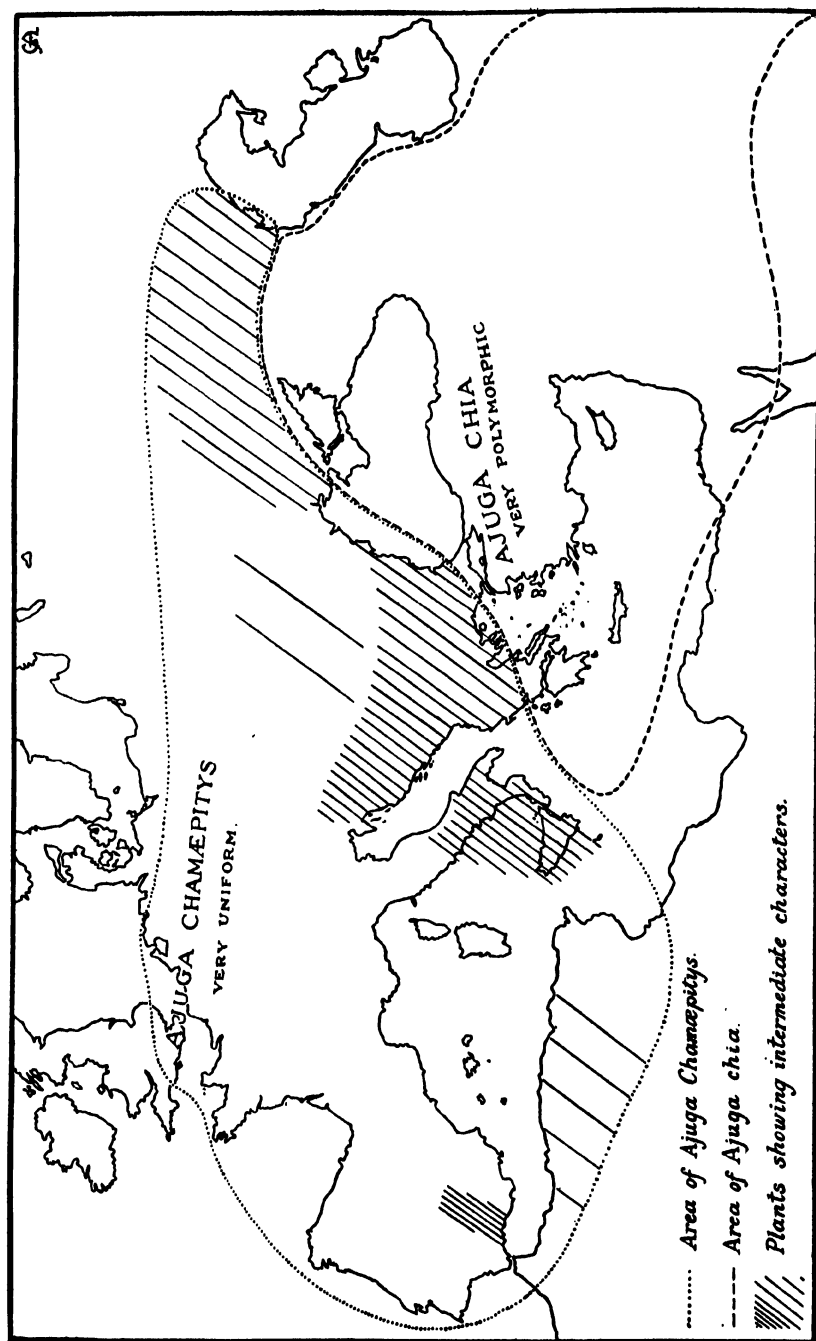
the floral characters used to distinguish *A. Chamaepitys* and *A. chia* but does not give a full account of their geographical distribution. It is the purpose of the present paper to trace the changes in morphological characters of these two species—if such they be considered—throughout their distributional range.

British Isles. The species occurs locally in cultivated fields and broken ground, chiefly on chalk, in nine English vice-counties (Druce, *Comital Flora*, 244: 1932), excluding records, now considered doubtful, for seven additional vice-counties. It is limited to the south of England, mainly to the south-east and south centre. The plants have mostly slender, presumably annual root systems, though the plant sometimes over-winters (*Little* 783, near Tingley Wood, Herts.). The stems remain simple or branch usually low down and sometimes profusely. The plants are always hairy and generally distinctly shaggy. The leaf segmentation is usually deep and the segments narrow, 0.5–1.5 (rarely 2) mm. broad. Individual, especially lower, leaves are sometimes only slightly lobed and are then broader. The flowers rarely exceed 1 cm. in length (though material up to 12.5 mm. has been measured) and are always shorter than the subtending leaf. The nutlets are 2.5 mm. long and reticulately pitted. The British material examined can be considered to be uniform apart from fluctuations¹.

France and Belgium. *A. Chamaepitys* is widely distributed in western Europe and the plants examined are mostly very similar to British material, though individuals are sometimes rather more robust with broader lower leaves. The root system is apparently annual and the flowers are small and much exceeded by the inflorescence leaves. The nutlets are definitely reticulately pitted.

Central Europe. *A. Chamaepitys* is probably not native in most of its recorded localities in Germany (see Hegi, *Illustr. Flor. v. Mitt.-Eur.* v, 4, 2539: 1927). In Switzerland it is fairly widespread though local in its occurrence and most abundant in the west. The Swiss and German specimens seen agree with those of western Europe (excluding Spain). In the Austrian lowlands and in the southern parts of Austria and northern parts of Yugoslavia the species is common. The nearly glabrous variety and one more hairy than the common plant (f. *hirta* Freyn) occur sporadically. In Czechoslovakia the species is rare, but

¹ Strictly, fluctuations are variations due to reactions of one genotype to different environmental conditions. Rather frequently one extends the use of the term to include variations in characters caused by the interaction of one or a group of genes with a varying external environment. The plasticity of a species is described by the range of the fluctuations of its biotypes.



from the little material seen taxonomically typical. In Hungary most of the material seen belongs either to the var. *glabriuscula* Holuby or to the var. *grandiflora* Vis. In both these varieties the nutlets are reticulately pitted and 3 to 3.5 mm. long. In the latter the corollas attain to 18 mm. in length and in one or two specimens the transverse corrugations of the nutlets become conspicuous. From Austria and Hungary southwards and south-eastwards the species becomes more polymorphic, on the whole larger flowered, and approximating to *A. chia*, a species which does not occur outside the Mediterranean region. Freyn (*Oester. Bot. Zeits.* xxvi, 408: 1876) ranges plants, seen by him, mainly from Austria, Hungary, Croatia and Istria, into three "groups" on their corolla size: *parviflora* 5-7 mm.; *media* 8-9 mm.; *grandiflora* 10-12 mm. Freyn's measurements appear to be rather understated, while Beck's (*Flor. v. Nieder-Oester.* II, 1023: 1893) statement that in Lower Austria flowers measure from 10 to 25 mm. in length indicates a maximum size not seen in any European material of *A. Chamaepitys* or *A. chia* preserved in the herbaria consulted. The figure 25 is possibly a slip or misprint for 15.

Italy and Sicily. Most of the specimens available for study agree with French and other western European plants, but sheets which must be classified as var. *grandiflora* have been received from Italy and Sicily, while the var. *glabriuscula* appears to be rather common in Sicily. One or two of the "*grandiflora*" specimens have nutlets with markings verging towards the transversely corrugated type of *A. chia* and about 3 mm. long, and the root stocks and roots are stronger and more woody than in typical *A. Chamaepitys*. The "forma *hirta*" Freyn is also recorded from the area (Fiori e Paoletti, *Flor. anal. d' Ital.* III, 8: 1903).

North Africa. Specimens with flowers approximating in size to the var. *grandiflora* Vis. have been seen from Morocco and Algeria. They have definitely reticulately pitted nutlets. Battandier and Trabut, *Flor. de l'Alger.* 715 (1890), record the species from a number of localities and also give *A. chia* as occurring in the Khreider, but no specimens have been seen and the record is doubtful. Little material is at present available for study.

Bessarabia and South Russia. The small quantity of material available from this area has flowers ranging in size from those of *A. Chamaepitys* var. *grandiflora* to those of *A. chia*. The leaves agree with those of British material and where roots are present the duration appears to be annual. The nutlets are reticulately pitted or show

markings intermediate between those of typical *A. chia* and typical *A. Chamaepitys*, and are 3–3.5 mm. long.

Crimea. The only two sheets examined have nutlets transversely corrugated and 3 mm. long and flowers mostly as long as or longer than the bracts. These specimens may be placed under *A. chia*.

Iberian Peninsula. There is little material available for dissection but what there is indicates a rather wide range of polymorphism. The plants tend to be large, the leaf segments remain narrow and the flowers small. Material under *Reverchon* 401 from Ronda has nutlets distinctly transversely corrugated and 2.5 mm. long, but though the corollas are faded they probably did not exceed or much exceed 10 mm. in length. A second sheet under the same number (*Reverchon* 401) but from Grazalema has its nutlets reticulately pitted and 3 mm. long. The Ronda material is *A. Chamaepitys* var. *suffrutescens* Willk. (in *Oester. Bot. Zeits.* xli, 53: 1891) and the plant is said to be perennial. The importance of the material, however, lies less in the name attached to it than in the interesting combination of characters which it shows—the nutlets being practically indistinguishable from those of eastern *A. chia*.

Greece. In Greece south of Thessaly the plants have mostly woody rootstocks and main roots and are probably perennial or at least over-wintering. On an average the leaves are somewhat shorter and their lobes a little broader than in British specimens of *A. Chamaepitys*. The corollas are large (20–24.5 mm. long, with one exception), and usually at full anthesis exceed in length the subtending bracts. The nutlets are either transversely corrugated or intermediate between this and reticulately pitted, and 3 to 3.25 mm. long. In other words, no Greek material which can be placed with certainty in *A. Chamaepitys* has been seen, and taxonomically the seventeen Greek sheets at Kew are classified as *A. chia*. One sheet demands special attention, that from Messenia: “in valle Nedontis supra Kalamata” (*Heldreich* 1466). This is named *A. chia* forma *intermedia* Heldr. and has flowers with corollas only 17 mm. long. The habit and the transversely corrugated nutlets, however, prevent it being placed under *A. Chamaepitys* and it is retained as a variation of *A. chia*.

Albania and Macedonia. The available material is poor but probably for the most part represents *A. Chamaepitys* var. *grandiflora*. One specimen from South Macedonia (*Harris* 73) may, however, be *A. chia*.

Thrace. Material from the Gallipoli Peninsula with corollas from 17.5 to 22 mm. in length and nutlets essentially transversely corrugated and 3.25 mm. long is classified with *A. chia*.

Bulgaria and Dobruja. Specimens at Kew are intermediate between *A. Chamaepitys* var. *grandiflora* and *A. chia* in character combinations involving habit, flower size, and nutlet markings. The nutlets are 2.75–3 mm. long.

Serbia. The only sheet at Kew (from Niš) is classified as *A. Chamaepitys* var. *grandiflora* and has the nutlets reticulately pitted and 2.75 mm. long.

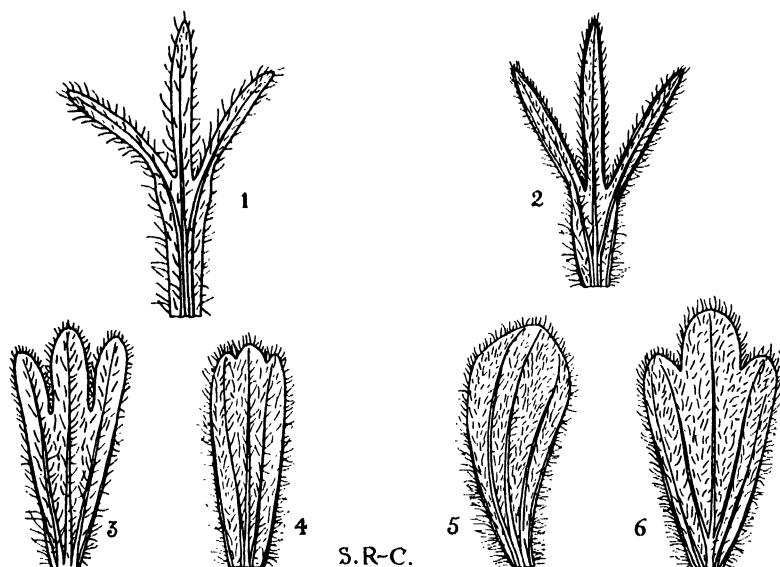
Bosnia and Hercegovina. The specimens examined are classed as *A. Chamaepitys* with corollas averaging 12–13 mm. in length and the nutlets reticulately pitted, 3 mm. long.

Dalmatia. In this area *A. Chamaepitys* varies considerably. Visiani described (*Flor. Dalm.* II, 222: 1847) the var. *grandiflora* from near Sebenico in the words "foliis floralibus florem subaequantibus." In addition, plants glabrous or nearly glabrous in all their parts occur apparently associated with hairy plants. These constitute the var. *glabra* Vis. *Flor. Dalm. Suppl.* 91 (1872). Visiani's description runs "caulibus puberulis, foliis calycibusque glaberrimis," and the type came from round about Stobreč, near Spalato. Unfortunately, in the same year, the name *glabriuscula* was published by Holuby in *Oester. Bot. Zeits.* XXII, 79 (1872), and probably this name antedates *glabra* Vis., and since it represents the same variation is retained here. The nutlets in Dalmatian material are 3 mm. long.

Istria. The specimens examined conform with *A. Chamaepitys* var. *grandiflora* with corollas about 15 mm. in length and nutlets reticulately pitted 2.5–3.25 mm. long.

Asia Minor, Syria, Palestine, etc. It is unfortunate that no material from the island of Chios (the type locality of *A. chia*) is available for study. There can, however, be little doubt that material from Attica is rightly regarded as typical of the species. On this basis a considerable number of sheets from the western, southern and central districts of Asia Minor are referred to *A. chia*. These have corollas 2 cm. or slightly more in length, a moderately dense indumentum, a habit suggesting that the plants are biennial or even perennial, and nutlets from 3 to 4 mm. in length. Some plants with reticulately pitted and others with transversely corrugated nutlets occur in the area. Additional to such specimens as may be safely accepted as *A. chia* there occur others which diverge in one character or more to such an extent that they have been considered varieties worthy of name or even distinct though allied species. These are probably most easily considered by using Boissier's classification (*Flor. Or.* IV, 802–4: 1879).

A. chia var. *latiloba* Boiss. Type specimens from Mt Mesogis, Lydia, and other specimens from Gheidagh (*Heldreich*) have been examined. The plants are evidently found in high mountain habitats and are characterised by their broad leaves and leaf-lobes and the long soft indumentum of all parts. The corollas are the largest known in the species considered in this paper and measure up to 28 mm. in length. The nutlets are transversely corrugated and 3 mm. in length. Material from Cyprus (Mt Pentedactylos, *Sintenise et Rigo* 123) links this variety on to var. *tridactylites*. The variety was originally



Figs. 1-6. Leaves. 1. *A. Chamaepitys*, Gravesend, England. 2. *A. chia*, Kephissia, near Athens, Greece. 3 and 4. *A. chia* var. *latiloba*, Gheidagh, Asia Minor. 5 and 6. *A. chia* var. *latiloba*, Pentedactylos, Cyprus.

described as a distinct species, *A. mesogitana*, by Boissier (*Diagn.* 1, vii, 62: 1846).

A. chia var. *suffrutescens* Boiss. includes material with a strong woody stock and roots, usually markedly canescent indumentum, leaves generally not deeply divided, corollas averaging 17 mm. in length, yellow or more or less red, and the calyx teeth tending to be somewhat short relative to the size of the calyx as a whole. The nutlets are transversely corrugated, but sometimes the corrugations are shallow and very close together. The nutlets range from 2.5 to 3.5 mm. in length. The variety was described originally by Boissier as a distinct species, *A. palaestina* (*Diagn.* 1, xii, 92: 1853). There is no

hard and fast combination of characters separating this variety from others of oriental *A. chia*. Specimens placed in the variety are common from Palestine and Syria but have also been received from Asia Minor.

A. chia var. *tridactylites* was originally described as a distinct species, *A. tridactylites* (De Ging. ex Benth. Lab. 699: 1835), from Mt St Catherine, Sinai, and from the foot of Mt Lebanon, Syria. Material from Asia Minor, Cyprus, Syria, Palestine and Persia has been referred to this variety of *A. chia*, as it is made by Boissier (*Flor. Or.*). The plants have a distinctly woody stock, usually rather short aerial branches, a fair amount of indumentum, leaves and leaf-lobes rather broad, corollas 15–18 mm. long, nutlets transversely corrugated, 2.25–3 mm. long. From Cyprus and from Persia broad-leaved and broad leaf-lobed specimens link on the "variety" to var. *latiloba*. It is probable that the "variety" is of an ecotype nature and characteristic of particularly dry ground. Popow, in *Flor. Cauc. crit., Labiatae*, 11 (1916), gives numerous localities for *A. chia* from the Caucasus and has a variety, *pseudochamaepitys*, characterised chiefly by "nuculis subverriculosis." It is doubtful if this variation has much taxonomic value. Plants with very shallow corrugations or reticulations are not uncommon in various parts of the Nearer East. No plants from the Caucasus are accepted as *A. Chamaepitys* by Popow.

A. vestita Boiss. and the very closely related (if specifically distinct) *A. bombycina* Boiss. from Asia Minor (and probably slightly extending over the present southern and eastern borders of Turkey) are characterised by the dense long white indumentum. They are undoubtedly related taxonomically to *A. chia* and are probably derivatives from it. With our present knowledge they are best kept specifically separated from *A. chia*, though they represent an extreme divergence in the development of a lanate indumentum from the commoner *A. chia* type.

A. laevigata Boiss. is also tentatively kept distinct. It has a fairly compact area of distribution in southern Asia Minor and Syria. The plants are usually robust, the lower parts of the stems being suffruticose and bare of leaves or nearly so. The leaf segments are narrow, the flowers large (corollas up to 25 mm. long), and the nutlets transversely corrugated, 2.75–3 mm. long. In some characters the species parallels *A. Chamaepitys* var. *glabriuscula*. What, from the single available sheet, appears to be a variation with the development of long distinct hairs on most parts, is *A. comata* Stapf (in *Denkschr. Acad. Wien*, 1, 50: 1885), collected "in agro Ecbatanensi (Media)."

This concludes a concise survey of the material of the *A. Chamaepitys-chia* affinity. The following facts are obvious:

1. There is no absolutely sharp line of morphological differentiation between the plants considered in this paper. On the basis of structure alone no valid objection could be found to considering them all as variations within one polymorphic species. Though individual specimens showing one or more characters extremely developed are markedly different from other individual specimens showing other character extremes, the rich material already filed at Kew shows that all intermediate grades occur between the extreme characters and character combinations.

2. There is, however, a striking generalised change in the *A. Chamaepitys-chia* population as one proceeds from north-west Europe to the Nearer East. One can trace a more or less gradual transition of characters as follows:

Duration.

Annual or biennial → biennial or perennial.

Habit.

Herbaceous → suffruticose.

Combined leaves and bracts.

Deeply divided into relatively long slender segments → shorter and broader segments or lobes.

Flowers.

Small (corollas 10-12.5 mm.) → large (corollas up to 28 mm.).

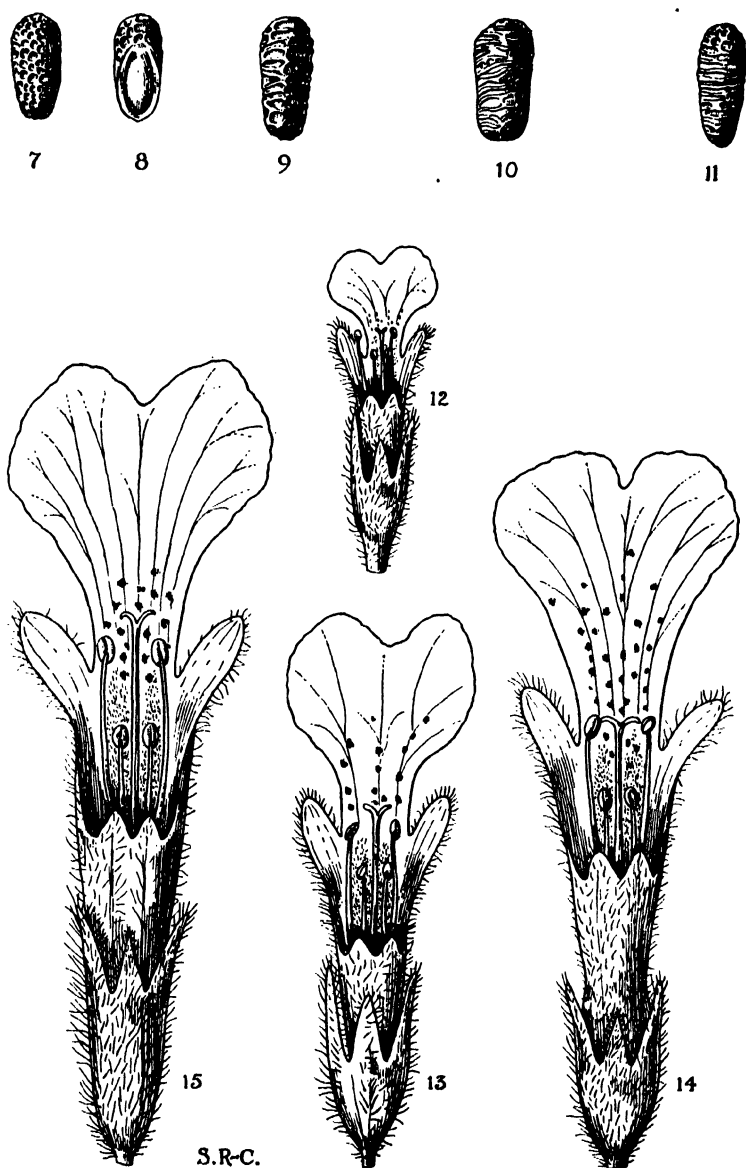
Nutlets.

Reticulately pitted, 2.5 mm. long → transversely corrugated, up to 4 mm. long.

3. The greatest morphological diversity is found in the Nearer East (Orient in the sense of Boissier), especially in Asia Minor.

4. Parallelism, probably due to independent mutations, is found in indumentum characters. Thus *A. Chamaepitys* var. *glabriuscula* occurs in Sicily and Italy and in Hungary. *A. laevigata* occurs in Asia Minor and Syria, and, though possessing the large corollas and transversely corrugated nutlets of *A. chia*, parallels in the absence or almost complete absence of hairs the var. *glabriuscula* of *A. Chamaepitys*.

There can be no dogmatic taxonomic conclusions based on a



S.R.C.

Figs. 7-11. Nutlets. 7 and 8. *A. Chamaepitys*, Dartford, England. 9. *A. Chamaepitys* var. *grandiflora*, Báhos, near Pest, Hungary. 10. *A. chia* var. *suffrutescens*, Wad el Kelt, Palestine. 11. *A. chia*, Greece. Figs. 12-15. Flowers. 12. *A. Chamaepitys*, Gravesend, England. 13. *A. Chamaepitys* var. *grandiflora*, Báhos, near Pest, Hungary. 14. *A. chia*, near Athens, Greece. 15. *A. chia* var. *latiloba*, Mesogis, Asia Minor.

study of herbarium material alone in this group of plants. If it be decided to unite the plants considered above as one species, then the name *A. Chamaepitys* must be used for the combined species. It seems, however, most reasonable, as it certainly is most convenient, not to make so "large" a species. Thus *A. laevigata* is fairly well definable morphologically and has apparently a continuous area of distribution. That it is morphologically linked on to *A. chia* by *A. comata* Stapf is certain, but the latter is only known from western Persia and may not really be a phylogenetic link. *A. vestita* and *A. bombycina* are more tentatively kept distinct from *A. chia*, the impression being obtained that they are "ecotypes" of very dry or exposed ground, and may ultimately be found to be linked with different stocks of *A. chia* in different localities.

For convenience of exposition it has so far been assumed that *A. Chamaepitys* and *A. chia* are two species, connected by plants showing intermediate characters or intermediate combinations of characters. In the absence of breeding and cultural experiments and without intensive and extensive field studies the following are the best reasons supporting this classification. In most of western and central Europe *A. Chamaepitys* is a relatively uniform morphological entity which taxonomically and phytogeographically must be given either a specific or a subspecific name. In Greece, and probably also the Aegean Islands, the large-flowered *A. chia* is (except for the "forma *intermedia*" of Heldreich) another distinct morphological entity which extends into Asia Minor and farther east. It is again difficult to avoid giving this a specific name, the more so because in Asia Minor, Syria, Palestine and Sinai variations, especially in habit, occur which are not paralleled in western material. The following is, therefore, the classification proposed:

A. Chamaepitys.

α *Chamaepitys* s.s. England, France, Belgium, Switzerland, Germany, Austria, Czechoslovakia, Italy, Northern and Central Balkan Peninsula, North Africa.

β *glabriuscula* Sicily, Italy, Hungary, Dalmatia.

γ *grandiflora* Sicily, Italy, Hungary, Northern and Central Balkan Peninsula.

δ *suffrutescens* Spain.

A. chia.

α *chia* s.s. Greece, Aegean Islands, Asia Minor, Caucasus, Persia.

β latiloba Asia Minor.

γ suffrutescens Asia Minor, Syria, Palestine.

δ tridactylites Asia Minor, Cyprus, Syria, Palestine, Sinai, Persia.

A. vestita Asia Minor.

A. bombycina Asia Minor.

A. laevigata Southern Asia Minor, Syria.

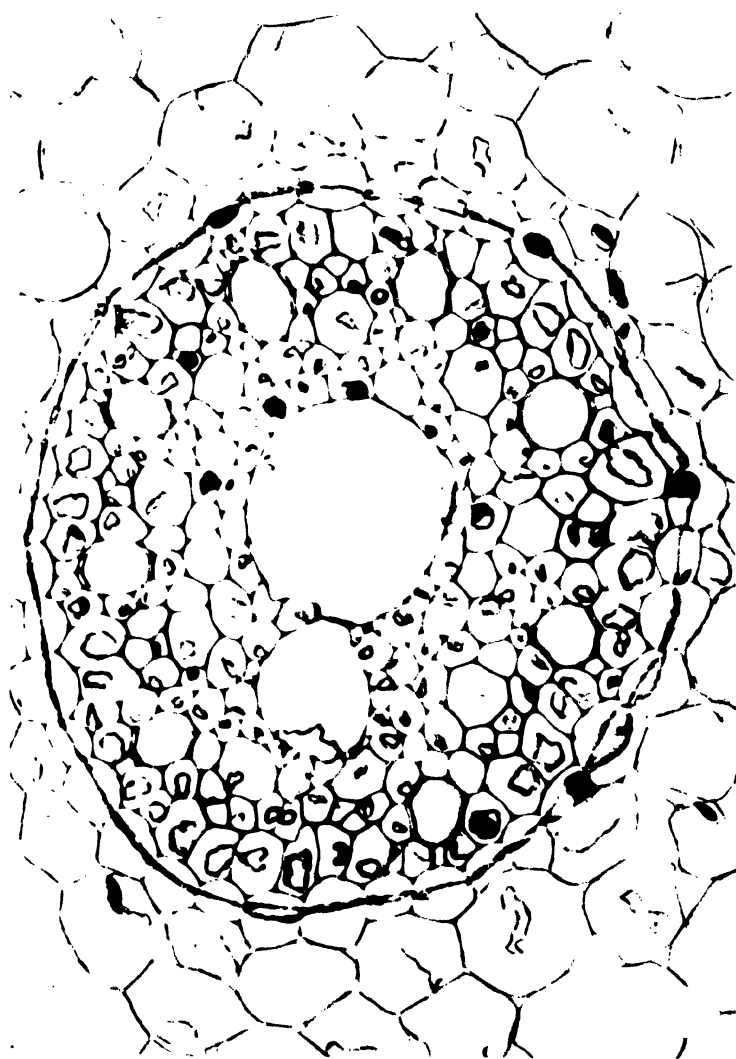
A. comata? Persia.

We have now to consider the possible history of the group. There can be no doubt that it is essentially Mediterranean in distribution. Not only is its greatest variation found in the Nearer East but the plants occur there in the most natural habitats. In north-western and central Europe and a part of the western Mediterranean Basin *A. Chamaepitys* is a weed of arable land, not of uncultivated hill slopes or the screes or rocks of mountains. Thus the evidence favours an extension of distribution westwards from an evolutionary centre near the Aegean or Levant. During this extension selection occurred more or less gradually, purifying the population in the direction of greater adaption to the conditions of weed vegetation in arable land. In some way not understood, but possibly gene linkage played a part, this resulted in smaller flowers in the population which succeeded, as typical *A. Chamaepitys*, in reaching the greatest extension in the north-west after the Ice Age. In the Nearer East the stock must be considered as a relatively old one. Many of the variations occurring there are obviously adapted to life under conditions approaching those of semi-deserts, i.e. they are xeromorphic in general habit and more or less definite ecotype selections. On the other hand such a segregation as *A. laevigata* suggests a mutational origin from the older *A. chia* stock.

An alternative theory, that *A. Chamaepitys* and *A. chia* are two species of independent origin which have extended, the former from the west and the latter from the east, to meet, say, in the Balkan Peninsula, is less likely than that outlined above. The available material does not suggest hybrid swarms in a particular meeting place of two or more species. Moreover, *A. Chamaepitys* and *A. chia* are morphologically much more closely related to each other than either is to any other known species.

SUMMARY

An account is given of the characters, variations and geographical distribution of *Ajuga Chamaepitys* and *A. chia* and of a few closely related species. It is shown that *A. Chamaepitys* is essentially a western and *A. chia* essentially an eastern plant, but that individuals and populations intermediate in characters and character combinations link up the two. It is suggested that the group arose in the Aegean and Levant areas and spread especially westwards and northwards, becoming less diversified by purification in the process of extension which was only completed, to its present extent, after the Ice Age.



BRYANT—A DEMONSTRATION OF THE CONNECTION OF THE PROTOPLASTS OF THE ENDODERMAL CELLS WITH THE CASPARIAN STRIPS IN THE ROOTS OF BARLEY

A DEMONSTRATION OF THE CONNECTION OF THE PROTOPLASTS OF THE ENDODERMAL CELLS WITH THE CASPARIAN STRIPS IN THE ROOTS OF BARLEY

By A. E. BRYANT

(With Plate I)

AMONG the sections prepared for microscopic examination, while working on the problem of the anatomical and histological differences which accompany the different types of root systems found when barley is grown in an aerated and a non-aerated culture solution, was the very interesting one shown in Plate I. This plate is a photomicrograph of a transverse section cut 25 mm. from the end of a root.

The killing and fixing agent was Karpenchenko's solution:

Mix equal parts of "A" and "B"

Stock solution "A"

Stock solution "B"

65 c.c. water

35 c.c. water

10 c.c. glacial acetic acid 40 c.c. 40 per cent. commercial formalin

1 grm. chromic acid

n-Butyl alcohol was used for dehydrating and imbedding.

In the process of killing and fixing all the cells were plasmolysed. The interesting thing about the plasmolysis is the position taken by the protoplasts of the various tissues with respect to their cell walls. It will be noticed that all of the protoplasts except those of the endodermal cells shrank away from the cell walls into the centre of the cell cavity. However, in the endodermis a very different situation is found to exist. Here the protoplasts shrank away from the tangential walls but not from the radial and transverse walls.

The position assumed by the protoplasts of the endodermis during plasmolysis is well known. But, nowhere in the literature was found such a striking demonstration as is shown by this photomicrograph. That the protoplasts of the endodermal cells are firmly embedded in the Casparian strip, in the roots of barley at least, is shown by their reaction to the type of plasmolysis involved here.

REVIEW

THE FOUNDER OF MODERN PLANT PHYSIOLOGY

Julius Sachs, der Begründer der neueren Pflanzenphysiologie, 1832-1897. By ERNST G. PRINGSHEIM. Jena: Gustav Fischer. 1932. Pp. xii and 302, with 13 plates.

Professor Pringsheim's biography of Sachs, written to commemorate the centenary of the great botanist's birth, is an able, thorough, and extremely interesting piece of work. After a brief but vivid sketch of Sachs' career (30 pages) there follow 100 pages of lucid description and critical discussion of his scientific work, arranged under 15 headings, illustrating Sachs' great versatility, and bringing clearly before the reader the enormous extent to which he was responsible for the foundations of modern botany. Then come excerpts—all that seemed worth publishing—from Sachs' decidedly disappointing literary remains: an account of his published books, as distinct from contributions to journals; and then a very interesting and informative chapter on the famous Würzburg Institute, with some account of the conduct of the teaching and research of Sachs and his pupils. More than a quarter of this chapter is occupied by Dr Scott's "German reminiscences of the early 'eighties," contributed to *The New Phytologist* in 1925, which Professor Pringsheim has rightly thought worth translating in full. This gives a particularly vivid description of the atmosphere and daily life of the Institute from an English point of view. Other chapters deal with Sachs' methods, concepts and theories and with the characteristics of his mentality; while the last is devoted to excerpts from letters, extending over 12 years, to his closest friend and former pupil, Hugo Thiel, who became a high official in the Prussian Ministry of Agriculture and had a great influence on the scientific development of that subject. A list of Sachs' published works and of the sources of the biography, and a useful triple index, bring the book to a conclusion.

Both the part which Sachs played in creating the modern science of botany, and the peculiarities of his character, by no means all of them amiable, are brought out very clearly. The author of this biography is no indiscriminating worshipper, though his respect and veneration for the great man are clearly very deep. His evaluations of Sachs' varied contributions to science are extremely useful, and, combined with his critical remarks on Sachs' often unfair judgments of people and of rival views, give a strikingly just and well-balanced picture of the character and achievements of his subject.

Julius Sachs was born in Breslau in 1832, the seventh of nine children, most of whom died young. His father was an engraver, certainly of real artistic nature and ability, but highly unpractical, so that the means of the family were always straitened. The mother was of peasant stock, but of refined and sensitive nature, and devoted to

her children: she early recognised the ability of Julius, and did what she could to give him a good education. Apart from the anxieties and hardships arising from lack of money, the family life was good and happy. The Sachsés lived partly in Breslau and partly in the neighbouring country, where the young Julius was from the first a lover of the nature around him. But by the age of 17 he had lost both his parents, and being without means was at a loss how to complete his education, so that he thought of becoming a sailor. In Breslau, however, Sachs' elder brother and then Julius himself had made the acquaintance of the sons of Purkinje, the eminent investigator of the physiology of vision, then Professor at Breslau University. The young Purkinjes were keen collectors of plants and animals, and when Sachs became an intimate frequenter of their house he got to know Professor Purkinje himself, who was a great experimenter and indeed one of the founders of modern physiology. This introduction to a higher grade of culture, free from perpetual anxieties and full of active scientific interests, proved decisive for the career of the highly gifted youth, who already, while he was at school, had studied the plants and animals of the neighbourhood, and among other things had written a paper on the river crab, illustrated with beautiful drawings from his own preparations, and later (1853) published in Prag.

When Julius was left destitute on the death of his parents, Purkinje, who in 1850 was called to Prag as Professor of Physiology, came to the rescue by offering Sachs the post of private assistant with free board and lodging and a salary of 100 Gulden. His duties were mainly to make microscopic preparations and to execute scientific drawings for Purkinje. Sachs stayed 6 years with Purkinje, and there can be little doubt that he must have learned a great deal from so able and experienced an experimentalist, highly gifted as he himself undoubtedly was. In later years he thought unwillingly of this period of his life, in which he seems to have been far from happy, though we have no direct evidence of the real nature of his personal relations with his employer. Purkinje was then in his sixties, and though even in old age he was said to be cordial and responsive in his intercourse with young people, he was a stern master in his own house. As years went on Purkinje became increasingly devoted to the propaganda of Czech nationalism, while Sachs was rather a pan-German. And it is more than probable, from what we know of his character in later life, that Sachs was a proud and difficult young man, conscious of his own genius and longing for complete independence. However that may be, and though he complained of the severity of his work for Purkinje—it was in fact 4 hours a day—Sachs found time to get through his "Abiturium" examination at the Gymnasium with great credit, and to begin his studies at the University. He was then, as at all times, an extraordinarily hard worker, giving very long hours and complete devotion to his subject. At this time he practised drawing from the antique in the Prag museum and even considered the possibility of becoming an artist. Fortunately, as his biographer remarks, he decided against this course, for if he had followed it we should merely have had another painter among many instead of a unique scientific investigator and philosopher. He made little use of botanical

lectures, but went instead to the original sources, and also worked at physics and mathematics by himself. He began experimental work on plants in his room in Purkinje's house in company with one of the sons and perhaps with Purkinje himself, and published a number of papers on the most varied botanical subjects in the Czech periodical *Živa*. He also studied philosophy, reading Locke, Leibniz, Hume, Kant and Herbart, under the influence of the Professor of Philosophy, Zimmermann, who, with the exception of one of his old teachers, Rumpelt, at Breslau, was the only man Sachs recognised as having taught him anything. "The sense for philosophy I had, but he (Zimmermann) put me on the right track," he wrote later on.

In 1856 Sachs left Purkinje, took his doctor's degree and in the following year became Privatdozent in Plant Physiology, the first there ever was. This was only managed after considerable difficulty, since the subject was not at that time recognised as a "Fach," and Rochleder, the Professor of Chemistry, though he himself worked at plant substances, said that Sachs would have to lecture on something else because the whole of Plant Physiology could be taught in 2 or 3 hours! The subject of Sachs' Habilitationsschrift was "Diffusion," but it was supported by a number of strictly botanical papers.

This was the beginning of Sachs' entirely independent activity, and at this time were laid the foundations of all the most important work of his life, to be developed during the next quarter of a century. He worked with the most intense activity, but stood extraordinarily alone, and saw very little of his botanical colleagues, though such men as Nägeli, Braun and Unger were at work and accessible. The most important contact he made was in 1857 with Wilhelm Hofmeister, the immortal discoverer of the unity in plan of the life history of bryophytes, pteridophytes and gymnosperms, at that time a Leipzig dealer in music! The acquaintance between these two ripened into a friendship most fruitful for both.

Sachs' work on water cultures, and its application to the purposes of agricultural chemistry, attracted the attention of the Professor of Zoology in Prag, Stein (the well-known investigator of Protozoa), who brought it to the notice of his former colleague Stöckhardt, the Professor of Chemistry at the Agricultural College at Tharandt in Saxony. Stöckhardt was greatly impressed, and as a result Sachs was invited to come to Tharandt as physiological assistant. Sachs took up his new post in 1859 and spent a happy and intensely active two years at Tharandt, where he could work in a much more congenial environment than at Prag with its extremely nationalistic Czech atmosphere. At Tharandt he experimented on the major problems of plant nutrition and also cultivated his outstanding powers of lecturing for which he later became famous. He also now saw a good deal of his colleagues at Dresden and Prag and of his friend Hofmeister at Leipzig. His reputation rapidly grew, and in 1861, after a suggestion, which came to nothing, that he should organise an Agricultural Section at the Chemnitz Polytechnic on the lines of the Institute at Tharandt, he went to the Poppelsdorf Agricultural College at Bonn as lecturer in Botany, Zoology and Mineralogy, after marrying a Prag lady to whom he had been engaged for some years.

At Bonn Sachs found good though modest conditions for his work, and his botanical lectures were so successful that after two years he was excused from lecturing on any subjects other than Plant Physiology and Agricultural Plants. During the six years he spent at Bonn his numerous publications, mostly contributed to *Flora*, really laid the foundations of the modern science of plant nutrition. In 1865 he published the *Handbuch der Experimentalphysiologie der Pflanzen*, already begun at Tharandt, and it was this book that securely established his reputation among his colleagues as the leading plant physiologist. At Bonn too he had his first two serious pupils, Kraus, who succeeded him, and Thiel, who became his closest friend. The accommodation was however very restricted, the stipend small, and he was disappointed in not being chosen to follow Schacht, who was Professor at the University of Bonn. This post went to Hanstein, one of the best microscopical observers of his time, and well known for his "three-layer" doctrine of the meristem of the higher plants. Sachs was accordingly very glad to receive a call to Freiburg in Breisgau, as successor to De Bary, and there he went in 1867. This was his first "ordinary" professorship, but it was by no means a wholly satisfactory position for his original work. The Botanic Garden was quite inadequate, the income small, the students few. Sachs seems to have spent his time at Freiburg almost entirely in writing the first edition of his famous *Lehrbuch der Botanik*, published in 1868, of which four editions appeared. In the autumn of 1868 he moved to Würzburg as the successor of Schenck, who had been called to Leipzig.

Here at Würzburg Sachs found somewhat better conditions for his work, conditions which he was able to improve very considerably as the years went on and his fame steadily increased. He received many attractive offers of chairs at famous universities—Heidelberg, Bonn, Vienna, Berlin, Munich—some with much larger incomes than he was getting at Würzburg; but all these he rejected, generally on the ground that the heavier duties of teaching and administration would interfere too seriously with his researches. And at Würzburg he remained until his death in 1897. The University and the Bavarian Government were by no means wanting in their recognition of Sachs' genius and unique position in the botanical world. The Garden and Institute were enlarged and improved, so that Sachs eventually had at his disposal facilities for physiological research which at that time existed nowhere else: a large new lecture theatre was built, and his stipend was greatly increased, so that by 1873 he was able to write that he no longer had the money troubles which had weighed upon him ever since he married. Titles and honours, which he never sought, but which also he did not despise, were conferred upon him. His brilliant lectures—illustrated by equally brilliant drawings done on the blackboard or in charcoal on sheets of paper while he talked—attracted students from other Faculties and also people of standing in the town. Many students not only from Germany but from abroad came to work under him.

Of the foreign students the English were the most numerous. Sachs' reputation grew very rapidly in this country during the

'seventies, largely owing to his *Lehrbuch*. It was the time of the biological renaissance in England: Darwin's work was of course exercising an immense influence, masses of new facts of structure and development were being discovered and interpreted in the light of the theory of descent. Huxley was Darwin's prophet, interpreting his work and spreading his influence. He was himself a skilled anatomist and morphologist as well as an eloquent lecturer, and at South Kensington he organised for the first time biological teaching with practical work. On the botanical side he found an able and enthusiastic coadjutor in Thiselton-Dyer, who translated the *Lehrbuch* and thus introduced Sachs' work to a wide circle of English and American readers. Apart from Darwin the great stimulus to biological progress came from Germany, where the new biology—"scientific" biology as it was often called—was being created by a brilliant group of investigators. The interest of young Englishmen of ability was aroused, their imaginations and enthusiasm excited; and they began to seek in Germany the sources of the new knowledge, to learn there from the great masters of the subject.

On the botanical side Sachs' institute at Würzburg became, as Scott says, the Mecca of the young English botanists—Francis Darwin, S. H. Vines, Marshall Ward, D. H. Scott and Walter Gardiner. Vines, at Cambridge, had become an enthusiastic exponent of Sachs' teaching and in 1877 went to Würzburg to work and to learn how to establish a laboratory for plant physiology. He became a close friend of Sachs, the correspondence between them lasting for 20 years till the latter's death in 1897. In 1881 Sachs even suggested that Vines should take a Professorship at Berlin.

In 1870, 1872 and 1874 the second, third and fourth editions of the *Lehrbuch* appeared, each extensively revised and enlarged; and it was this work, more than any other, which spread Sachs' fame throughout the world of botanical science. It was indeed the most potent instrument in the development of modern "scientific" botany throughout Europe, and later in the United States. A French translation by van Tieghem appeared in 1874, and an English one by A. W. Bennett and Thiselton-Dyer in 1875, with a second edition in 1882.

In 1875 Sachs published the *Geschichte der Botanik vom 16 Jahrhundert bis 1860*, i.e. approximately to the time when his own fundamental researches began. An English translation by Garnsey and Balfour appeared in 1907. This book bears the conspicuous imprint of Sachs' characteristic genius for broad, well-balanced philosophical survey.

In 1882 appeared the *Vorlesungen über Pflanzenphysiologie* (a second edition in 1887), which was translated into English by Marshall Ward as *Lectures on the Physiology of Plants*. It was this translation, read when it appeared in 1887, that first attracted the interest of the present reviewer, as a boy, to scientific botany. The philosophical grip and mastery of the subject, the power and sweep of the exposition, were outstanding qualities which could not fail to make the deepest impression.

These three works, with the earlier *Experimentalphysiologie*, were all that Sachs published in book form. Pringsheim gives a list of 125

separate contributions to periodical publications, extending from 1853 to 1896. During the Würzburg period most of Sachs' more important work together with that of his pupils appeared in the *Arbeiten des botanischen Instituts in Würzburg*, of which three volumes appeared (1871-4, 1878-82, 1884-8).

Sachs' genius, which gave him his commanding influence on the development of modern botany, rested on a rare combination of characters. In the first place he possessed in very high degree the prime quality of a great investigator, the urge to go direct to nature and find out for himself; secondly he was a great experimentalist, with the ability to devise and construct his own apparatus, usually of the simplest kind, but essentially well fitted to obtain answers to his questions. Added to these powers he had the true philosophical mind which always and everywhere seeks the general significance of particular phenomena, never allowing itself to be overwhelmed with mere detail. And finally he had the spirit of the creative artist who aims at constructing an artistic whole from the materials with which he works. This it was which gave his writings and lectures, apart from the substance, their peculiarly attractive quality.

Pringsheim calls attention to an interesting and significant characteristic of Sachs' original drawings, so many of which were copied into smaller text-books in the last quarter of last century and became the sources of the notions of plant structure imbibed by innumerable students of botany. His skill in drawing was very highly developed: the original power, shown while he was still at school, was probably inherited from his father; but in his drawings from the microscope he never attempted to represent each individual cell exactly as it appeared. He always tried to seize the characteristic appearance of the cells of a tissue and to represent that. The consequence is that the great majority of his drawings might well be labelled "somewhat schematised." This procedure is excellently adapted for teaching purposes, but of course it has its drawbacks, since it may very well ignore features of which the draughtsman does not realise the importance. Later on the opposite tendency became dominant in botanical drawing from the microscope and was expressed also in the increasing use of photography for representing anatomical structure. "Truth to nature" was often purchased at the cost not only of aesthetic satisfaction but of intelligibility, a development for which Sachs would have had nothing but contempt.

Great and commanding figure as Sachs was he certainly had the defects of his qualities. With thoroughly justified confidence in his own powers and insight he could not tolerate opposition, and when once he had adopted a theory or taken up a point of view he would sometimes defend it with tenacity and obstinacy in the face of the most convincing evidence to the contrary. He often grossly underrated and even abused work with which he was not in sympathy. Thus he called Pfeffer's great text-book of plant physiology "a mass of undigested facts"; and he wrote of Strasburger's important work on the *Leitungsbahnen* (1892): "I feel doubly the decline of botany when I think of the time when I worked with Hofmeister, Mohl and Hanstein, and when a Strasburger, who has no notion of experimen-

tation, would certainly not have dared to write a book on sap movement weighing a kilogram, without knowledge of the literature and without any preparation in physics." It is to be noted that Pfeffer and Strasburger were (with Goebel) the leading German botanists of the period, i.e. about 1880 to 1900, immediately succeeding that of Sachs' greatest activity. But much of Pfeffer's physiology was uncongenial to Sachs, and Strasburger dismissed Sachs' imbibition theory of the ascent of sap. Sachs showed indeed in exaggerated form an unamiable trait not uncommon in great masters of science or philosophy—bitter hostility towards pupils who came to disagree with him or to develop views he was unable to accept.

Perhaps the most striking example of Sachs' want of balance in the critical judgments of his later life is seen in his attitude towards Charles Darwin. At first he received *The Origin of Species* with enthusiasm, and was greatly impressed also by other works, for instance *Insectivorous Plants*, but gradually he became more and more critical and reserved, till his final attitude was one of almost unqualified condemnation. The following theses (freely translated here) were found among Sachs' literary remains under the title of "The significance of Darwinism for Botany":

1. The morphological natural system was complete in its main outlines before Darwin's *Origin*, especially the governing relations of Phanerogams to the Higher Cryptogams established by Hofmeister.
2. Morphology and the structure of the natural system were not advanced by Darwin in any way.
3. Methods of research retrogressed as a result of Darwin's influence.
4. Scientific style was degraded by Darwin: his anecdotal method relaxed the strictness of scientific thought.
5. Anatomy and experimental physiology were not advanced, but progressed quite independently of Darwin, since he himself could neither make microscopical observations nor could he experiment.
6. So-called "biology" was advanced by him but to the injury of strict science.
7. The service which Darwin rendered was in respect of interest in the non-constancy of species, evidence for which he collected from the literature.
8. The theory of descent is erroneously ascribed to him: the real theory of descent contradicts the principle of selection.

The gross inability exhibited in this summary to recognise and appreciate the real nature of Darwin's genius and achievement needs no emphasis. But some consideration of Sachs' psychology enables us to understand, at least partly, how he came to pronounce so unjust and at the same time so unequivocal a verdict. As Pringsheim points out, the whole mentalities of Darwin and Sachs were polar opposites. Darwin was first and foremost a naturalist, who was forced to a belief in the mutability of species, and hence to the general doctrine of descent with modification, as a result of innumerable observations of animals and plants, supplemented by extensive reading in the literature of breeding. When the idea of natural selection through the struggle for existence came to him he saw in this process a *vera causa* of the modification of species and thus an explanation of "evolution." The whole of his later experimental work was that of a naturalist turning to experiment to elucidate the "mechanisms" he observed in

nature, the adaptive relations of organisms to the external world. Sachs, on the other hand, was first and foremost a philosopher, who desired to discover order in the variety of structure and function of the living organism. In the early days at Prag, while he was still Purkinje's assistant, he began to make a general survey of the structures and functions of plants, basing his investigations of function, so far as he could, on the physics and chemistry of the day, but always with the urge of the philosopher rather than that of the naturalist. At Prag, Tharandt and Bonn he developed this field in his own researches, and proceeded to expound the results in the *Experimentalphysiologie* and the *Lehrbuch*; at Würzburg various parts of the field were further exploited by himself and his pupils. Throughout his work the philosophical and the artistic impulses are everywhere apparent in his constructive exposition. On the experimental side Sachs was a "laboratory man," Darwin was not. Sachs was a professional philosophical botanist, Darwin was a gifted amateur of genius; though both were extraordinarily hard and persistent workers exclusively devoted to their science. So far as such generalisations are permissible it may be said that the two men were respectively typical of the English and of the German genius. Darwin's method and thought were discursive, though his work was in the interests of a vast generalisation; Sachs' were unitary, in spite of the diversity of the material with which he worked.

As Pringsheim says, in Darwin we have a thought-construction of enormous dimensions: proof is piled on proof and the individual building stones are not always sufficiently firm. But in spite of some gaps the logical structure has held firm, though it has been attacked by innumerable opponents, and though the facts are now to some extent differently interpreted. Sachs, starting from experiment, always sharply distinguished the proved from the supposed. His whole habit of mind made him extremely critical of Darwin's methods: and with the mode of approach to biology of the English naturalist his entire mentality was quite unsympathetic. By no means all of his individual criticisms are unfounded, though some of them have much more force against Darwin's superficial followers than against Darwin himself. But his summing up is almost ludicrously unjust.

For the root of this essential unfairness we must look not only to Sachs' type of mind, but also to his faults of character and to the weaknesses of his affective psychology. Sachs was not only austere in his thought and ideals, he was intellectually proud and obstinate, in the strongest contrast to Darwin's humility and readiness to admit himself wrong. Sachs certainly suffered much hardship from straitened means in early life, from the continued illness and final collapse of his wife, who had to go to an asylum in 1880, and from increasing ill-health and weakness in his later years; but he received the fullest recognition and abundant honour from his contemporaries, and yet he was full of complaints, by no means justified, of people and things. He was indeed extraordinarily bitter in his whole attitude towards the world during the last 20 years of his life. Untraceable influences in his childhood may have been partly responsible for this, and partly perhaps his long dependence on Purkinje.

We must indeed regretfully recognise that these defects rather seriously mar an otherwise magnificent mind and character. For when all is said Julius Sachs was a very great man, of commanding power and of noble and single-minded devotion to his science. His influence on botany in the nineteenth century was comparable, though on such different lines, with that of Linnaeus in the eighteenth; and we shall scarcely look upon his like again.

A. G. TANSLEY.

THE NEW PHYTOLOGIST

VOL. XXXIII, No. 4

12 OCTOBER, 1934

STUDIES ON THE RESPIRATION OF CON- JUGATING *SPIROGYRA* WITH SPECIAL REFERENCE TO FAT METABOLISM

By N. L. PAL, M.Sc.

University of Allahabad

(With 17 figures in the text)

CONTENTS

	PAGE
Introduction	242
Material and method	242
A. Collection and treatment of the material	242
B. The apparatus	243
C. Source of light	247
Section 1. Respiration of <i>Spirogyra</i> in different stages of con- jugation	248
A. Experimental results	248
(a) Respiration of conjugating <i>Spirogyra</i>	248
(b) Oil and starch content in conjugating <i>Spirogyra</i>	252
(c) Diastatic and lipasatic activity in <i>Spirogyra</i>	253
B. Discussion	254
(a) Intensity of carbon dioxide emission at different stages of conjugation	255
(b) The oxygen intake in conjugating <i>Spirogyra</i>	256
(c) The respiratory quotient in conjugating <i>Spirogyra</i>	257
(d) Substrate used for respiration in conjugating <i>Spirogyra</i>	258
(e) Fate of oxygen taken in	259
(f) True index of energy released in conjugating <i>Spirogyra</i>	260
(g) Oil reserve in <i>Spirogyra</i>	262
Section 2. Effect of light on physiology of conjugation	263
A. Experimental results	263
B. Discussion	265
(a) Effect of light on respiration in <i>Spirogyra</i>	265
(b) Assimilation of <i>Spirogyra</i> in absence of external supply of carbon dioxide	267
(c) Assimilatory quotient and influence of light on oil for- mation in <i>Spirogyra</i>	268
(d) Influence of light on conjugation in <i>Spirogyra</i>	269
(e) Economics of oil metabolism in <i>Spirogyra</i>	270
Summary	271
References	272

INTRODUCTION

WHILE working on the respiration of *Spirogyra* in one particular experiment rather low respiratory quotient values were obtained, though normally the ratio found was unity. A careful scrutiny of the experimental procedure revealed nothing that could possibly explain the matter.

An examination of the filaments used in this experiment showed that together with normal filaments there were some conjugating filaments mixed in the sample. The peculiar R.Q. was therefore attributed to the presence of conjugating filaments and the following experiments on the gaseous interchange in *Spirogyra* were performed.

The results are discussed in two sections. Section 1 deals with the respiration of *Spirogyra* in different stages of development and allied problems, while Section 2 deals with the effect of light on the interchange of gases in the same organism.

A search through the available literature showed no record of respiration in conjugating *Spirogyra*.

MATERIALS AND METHOD

A. Collection and treatment of the material

All the experiments were performed with *Spirogyra*, except two that were performed on *Sirogonium*. The organisms were collected locally from two adjacent ponds.

The *Spirogyra* collected for the experiments consisted probably of more than two species. But in most cases they were collected from a single spot, and in a single collection one species generally predominated. Sometimes a few other organisms were found mixed with the *Spirogyra*, but cases where they were abundant were rare. Collections thus contaminated with other filaments were always discarded.

The collected filaments were brought to the laboratory, placed in a flat dish of filtered pond water and kept in the light near a glass window for 2 days, previous to an experiment. Before an experiment was started, the filaments were examined under a microscope to note their condition. If they were not healthy, or if the filaments were contaminated with other organisms, the experiment was not started but a fresh collection was made.

The filaments were carefully washed in distilled water, changing the liquid several times. They were then placed in the apparatus for

20 min. without taking any readings, to allow the plants to get adapted to the new environment. Then the experiment was started in the same liquid which was previously boiled and cooled in an atmosphere devoid of carbon dioxide.

B. *The apparatus*

Winkler's method of estimating oxygen has been widely used for experimenting on the gaseous interchanges of water organisms. A difficulty arises, however, from the fact that the amount of oxygen dissolved in a sample of water is greatly affected by even very slight contacts with the atmosphere.

A very simple apparatus specially suited for biological experimentations was described by Osterhout and Haas⁽¹⁵⁾ in 1917. Although the difficulty of estimating oxygen is removed the apparatus is not an ideal one. In the first place, it does not allow the organisms a supply of continuously flowing liquid, which is essential for an experiment continued for a long time. In the second place, their method of introducing T-tubes for consecutive readings not only makes the apparatus difficult to handle but also renders the calculations complicated. Moreover, introduction of additional T-tubes becomes necessary for estimations of carbon dioxide interchange.

In view of the above difficulties, the apparatus described below was devised which gave very accurate results.

The apparatus (see Fig. 1) consists of the following parts: the thermostat bath; the reservoir and plant chamber; and two sets of collection tubes.

The thermostat water bath (*A*) is provided with a burner and a mercury flame regulator to keep the reservoir (*B*) and the plant chamber (*C*), which are immersed in it, at a constant temperature.

The reservoir (*B*) is a glass vessel, the capacity of which depends upon the kind of experiment and its duration. For algal work a 5-litre flask or bottle is very convenient. The plant chamber is a small cylindrical vessel (*C*) about 6 cm. diameter and 3 cm. high. There are two tubes attached to the plant chamber, one coming from the reservoir and the other, which is a capillary one, reaching the collection tubes *D* as shown in the figure. Between the plant chamber and the tube *D* is inserted a small capillary tube *T* about 6 cm. long provided with two screw clamps (*a* and *b*), one at each end.

Each set of collection tubes consists of three parts, *D*, *E* and *F*, mounted on a stand, *D* and *E* being removable. *D* is the oxygen estimating tube about 9 cm. long and having a capacity of 50 c.c. It

has three tubes fused with it. The first is a capillary one at the top connecting the plant chamber, the second connecting the tube *E* beneath, and the third about 1.5 cm. diameter and 2 cm. long at one side. This side connection which serves as an opening for the introduction of chemicals is provided with a screw clamp (*c*). The connection between *D* and *E* is also provided with a screw clamp (*d*).

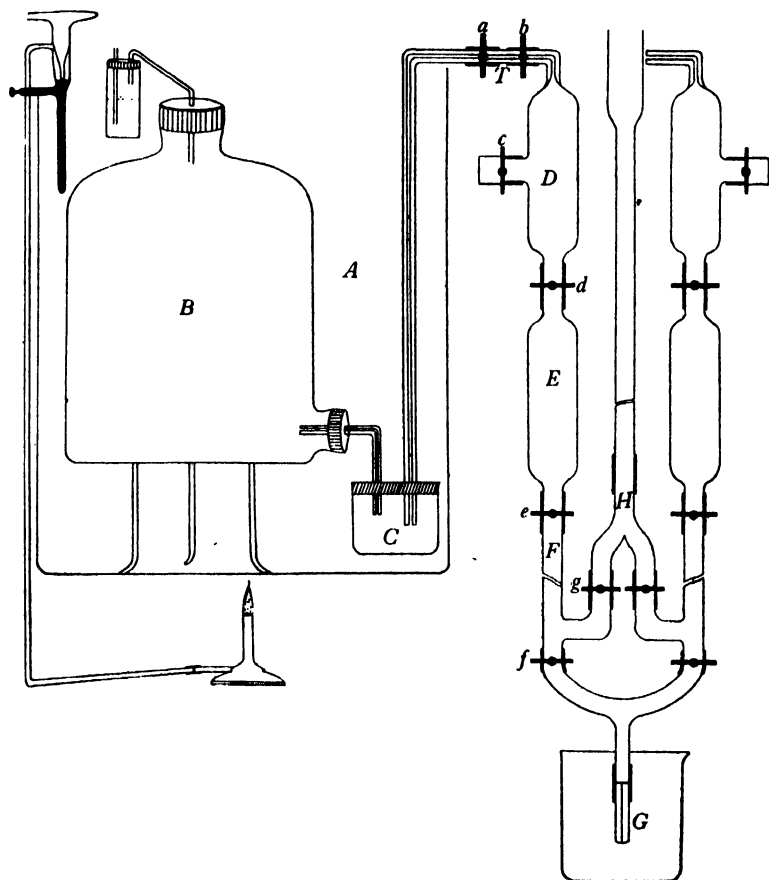


Fig. 1.

E is a tube similar to *D* and of the same size but without the side tube attached to it. *E* communicates with *F* beneath through the clamp (*e*).

F is a long tube about 30 cm. long and 1 cm. diameter with a side tube bent at right angles attached to it towards the lower end. The lower ends of *F* and the corresponding tube of the other set are

joined to two arms of a Y-tube through clamps (*f*). The third arm of the Y-tube is attached to a capillary tube *G*. This capillary tube is so graduated that if the tubes *D*, *E* and *F* are all filled with mercury and the clamps opened, about 100 c.c. of mercury will fall out in drops every hour. A tube of about 1 mm. bore, partially fused at one end to close the aperture to the desired diameter, is better suited for the purpose than a capillary tube of very fine bore. The tube thus fused is inserted in the apparatus with the open end outside serving as the outlet for mercury. This arrangement prevents the clogging of the bore and also spilling of mercury as it falls in drops.

The side tubes attached to *F* and its corresponding tube of the other set, referred to above, communicates through another Y-tube with *H*, which is a long tube serving as the filler and reservoir of mercury. These connections are also provided with clamps (*g*).

Manipulation of the apparatus.

The bath is first adjusted to the desired temperature. The reservoir bottle *B* is filled with the solution to be provided to the plants and the plants are placed in the chamber *C*. The stopper of the plant chamber is now tightly closed, taking care that the chamber is free of air bubbles. Both the reservoir and the plant chamber are now placed in position in the thermostat bath. In order that the reservoir may not communicate with the atmosphere directly, it is connected to a small bottle of water as shown in Fig. 1. Water is sucked out from *T* and allowed to flow out for some time to enable the plants to become adjusted to the new conditions, and then the clamp (*a*) is closed.

In the meantime the collection tubes are filled with mercury. This is done in the following manner. Mercury is poured in the tube *H* from above, the clamp (*g*) and its corresponding one being closed previously. By opening *g* carefully mercury is allowed to fill up the tubes *F* and the Y-tube beneath it. Clamp *f* and its corresponding one are now closed.

Mercury is now allowed to fill the tubes completely by opening *g* fully, taking care to close the side connection (*c*) previously. To avoid spilling the mercury, a tube bent upwards is inserted at *b*. When mercury reaches this tube, *b* is closed and the tube is carefully taken out.

T is now set in position. Clamps *a*, *b* and *f* are opened and mercury begins to drop from *G*, thereby drawing in water in the tubes *D* and *E* from the plant chamber.

When both *D* and *E* are thus filled with algal water and the

mercury level stands at a marked position in *F*, clamps *f*, *b* and *a* are closed. *T* is now taken out from the set and inserted in the other set which is kept ready previously filled with mercury. The necessary clamps are now opened so that mercury again begins to drop and algal water collects in the tubes of the other set.

From the volume of *D*, *E* and *F* up to the marked position and the time required to fill them with algal water the rate of flow is previously adjusted to about 100 c.c. per hour. As the total volume of *D*, *E* and *F* is also 100 c.c. one set of collection tubes works for about an hour, which time is utilised in estimation of oxygen and carbon dioxide in water collected in the previous set.

Estimation of oxygen and carbon dioxide.

Oxygen is estimated by Winkler's method as adapted by Osterhout and Haas. Clamp *d* is closed and the tube *D* is taken out from the stand. Reagents are now introduced in a manner identical to that of Osterhout and Haas.

A small glass tube about 3 cm. long and 1 cm. diameter is inserted at the side tube. Alkaline solution of potassium iodide is introduced in this tube until the level reaches up to the rubber tube attached to it. The free end is now closed by means of a clamp, taking care that no air bubble is introduced. The excess of liquid is poured off and the rubber is well washed first in running water and finally with distilled water. The clamp between *D* and the tube introduced is now opened.

A second tube is now introduced at the free end of the first and solution of manganese chloride is similarly introduced and the outer clamp closed. The clamp between the first and the second tube is opened and the apparatus inverted several times so that the reagents are well mixed. After washing the free end carefully as in the previous case the apparatus is left for about 15 min. to complete the reactions.

Then by means of a third tube about 2 c.c. sulphuric acid is introduced into the apparatus. When the precipitate previously formed is dissolved the whole solution is poured off in a beaker and the amount of iodine liberated is titrated against standard sodium thio-sulphate solution, using starch as indicator. From this the amount of oxygen dissolved in the sample of water is calculated.

The strengths of the reagents required depend upon the amount of oxygen dissolved. The following has been found to be quite suitable for experiments with algae:

- (1) 10 grm. of KI in 100 c.c. 36 per cent. NaOH.
- (2) 33 per cent. MnCl_2 .
- (3) H_2SO_4 diluted 1 : 1.
- (4) $N/100 \text{ Na}_2\text{S}_2\text{O}_3$.

Carbon dioxide is estimated by addition of excess of barium hydroxide solution, and then titrating the excess amount by standard HCl.

By means of a pipette, 20 c.c. of algal water is taken in a flask. The latter is provided with a stopper having two bores. Through the first bore is introduced a glass tube, one end of which is kept dipping in water in a beaker. Through the second, the end of a micro-burette (graduated to measure 0.05 c.c.) is introduced. A definite amount of $\text{Ba}(\text{OH})_2$, to which phenolphthalein has previously been added, is introduced into the flask from the burette. The flask is taken out and immediately connected to another micro-burette containing standard HCl. The excess of $\text{Ba}(\text{OH})_2$ is now titrated. The titrated liquid is kept in a stoppered flask and used as a standard for comparing the colour while taking the end-points in subsequent titrations.

The solutions used for algal experiments are

- (1) $N/150 \text{ Ba}(\text{OH})_2$.
- (2) $N/200 \text{ HCl}$ (standard).

C. Source of light

In the experiments with light an Osram 1000-watt electric bulb was used. The bulb was placed at a distance of 15 cm. from the plant chamber. Between the plant chamber and the bulb was placed a rectangular jar about 10 cm. wide filled with water to absorb the heat rays. The water in the jar was kept circulating by a connection from a tap and a siphon arrangement to the sink. The bulb was surrounded by a reflector.

The thermostat bath in which the reservoir and the plant chamber were kept immersed had a small glass window for the entry of the light. The chamber was placed with the flat surface at right angles to the light rays.

For the experiments in the dark the plant chamber was kept covered with a thick piece of black cloth.

The results obtained in all the experiments were referred to the dry weights of the plants. When an experiment was over the algae were taken out in a weighing tube and placed in an electric oven maintaining a temperature of 95–96° C. The weighings were made

after a period of 24 hours and were then constant. To this dry weight the activity of filaments was referred. In all the experiments the temperature of the bath was maintained at 35° C.

SECTION I. RESPIRATION OF *SPIROGYRA* IN DIFFERENT STAGES OF CONJUGATION

A. Experimental results

(a) Respiration of conjugating *Spirogyra*.

In all, six experiments were performed with *Spirogyra* and two with *Sirogonium*. These will be taken up one by one.

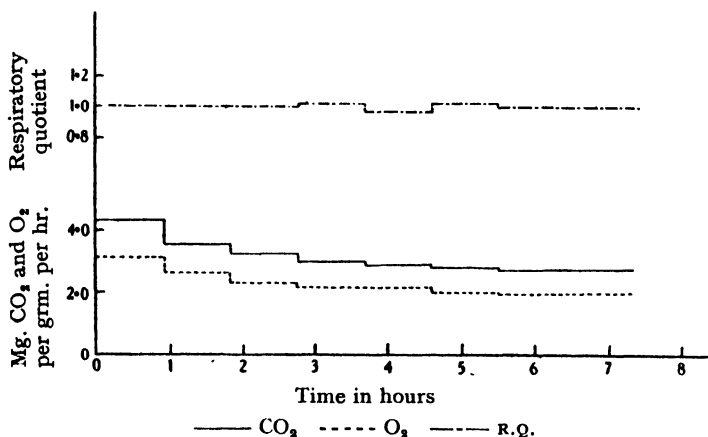


Fig. 2. Respiration of *Spirogyra* without conjugation.

Fig. 2 represents the respiration of normal *Spirogyra* filaments. The respiration intensity at the beginning of the experiment is 4.31 mg. carbon dioxide and 3.13 mg. oxygen per gm. (dry wt.) per hour. It gradually falls off to the lower values of 2.74 mg. carbon dioxide and 1.99 mg. oxygen at the end of the experiment. The respiratory quotient throughout the experiment remained near unity—the very slight departures being entirely due to experimental errors.

The algae employed in this experiment were healthy, fully green and slimy to touch, and remained so till the experiment was over. In this collection there was no cell conjugating or sending out conjugation tubes.

Fig. 3 represents the respiration curve of a mixture of vegetative and conjugating filaments. The conjugating filaments were roughly about 50 per cent. of the total quantity taken and were in a very

early stage of conjugation. The output of carbon dioxide was 2.72 mg. per hour, while the oxygen intake was as high as 4.90 mg. per hour. Because of the high oxygen intake the respiratory quotient came down to the low value of 0.5. The respiratory intensity, however, remained fairly constant throughout the experiment.

The rest of the experiments were performed with conjugating filaments alone. This was quite easily managed, as the filaments of

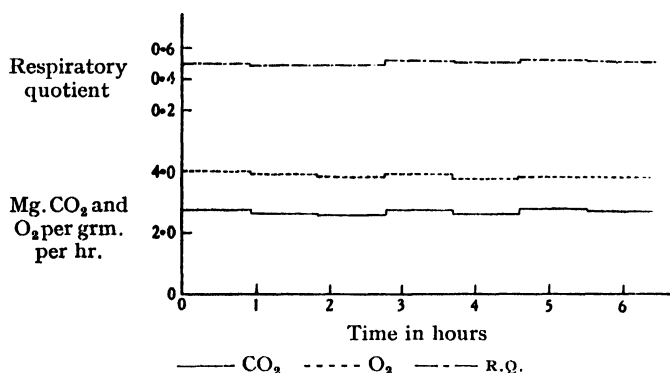


Fig. 3. Respiration of *Spirogyra*. 50 per cent. of the filaments conjugating.

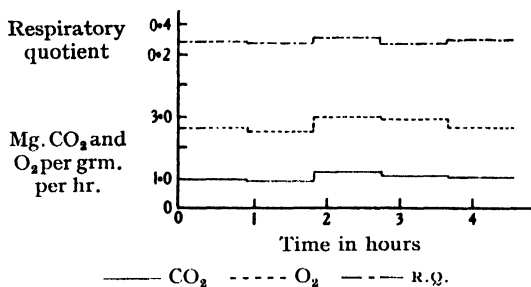


Fig. 4. Respiration of conjugating *Spirogyra*. Conjugation tubes formed but migration of the protoplasm not yet begun.

Spirogyra generally undergo conjugation simultaneously, leaving out very few vegetative filaments. The few normal filaments that were present originally in the sample were, however, carefully removed under a dissecting microscope before the experiments were started. It is to be noted here, however, that though generally a great number of cells of a filament take part in conjugation, there are almost always some inactive cells which fail to produce a conjugation tube, thus leaving a few vegetative cells in the filament.

Fig. 4 represents the respiration of filaments, the cells of which have already sent a good number of conjugation tubes. Very few

cases of passage of protoplasm from one gamete to the other had taken place in this collection. The chlorophyll bands in most cases were intact though a bit disorganised. The intensity of respiration was much lower, being only 0.97 mg. carbon dioxide and 2.57 mg. oxygen, which kept nearly level throughout. The respiratory quotient was as low as 0.27.

Instead of destroying the filaments used in the last experiment, they were placed in pond water in a well-lighted place at room temperature for 2 days. After the expiry of the period, an examination under the microscope revealed the fact that the process of conjugation had in the meantime far advanced and a great number of protoplasmic migrations and some zygosporic formations had taken place. These filaments were now put in the respiration chamber and the experiment started.

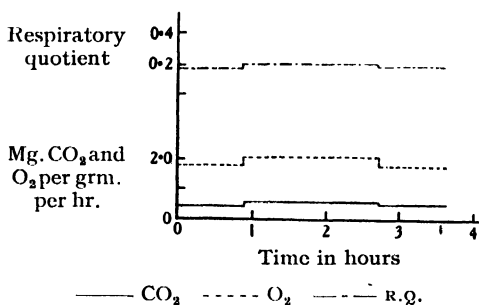


Fig. 5. Respiration of conjugating *Spirogyra*. Protoplasmic migration has begun.

In this experiment (Fig. 5) both the carbon dioxide output and the oxygen intake fell off to about 0.5 mg. carbon dioxide and 1.8 mg. oxygen. The R.Q. also fell to 0.2.

In the next experiment filaments at a very advanced stage of conjugation were employed. The content of all gametes had rounded off, and in the majority of cases the protoplasm of one had travelled to the other. Numbers of fully formed zygosporic forms were also present. The results are shown in Fig. 6. The carbon dioxide output and oxygen intake and the R.Q. all remained low as in the previous experiment.

Two experiments were now performed with *Sirogonium*, another member of the Conjugatae. The filaments were collected from the same pond and were treated in a similar manner.

Fig. 7 represents the respiration of *Sirogonium* filaments without any conjugation. The intensity is 3.21 mg. carbon dioxide and 2.33 mg.

Studies on the Respiration of Conjugating *Spirogyra* 251

oxygen. These do not show a falling tendency in agreement with the normal *Spirogyra* filaments. The R.Q. is near unity as in *Spirogyra*.

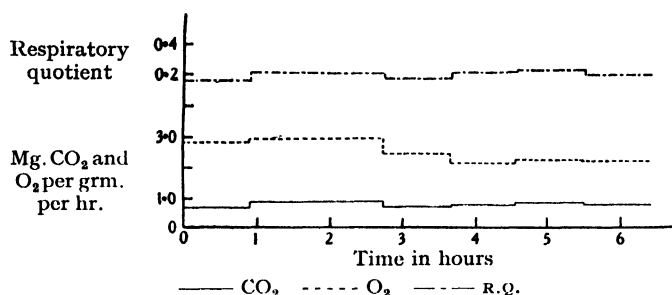


Fig. 6. Respiration of conjugating *Spirogyra*. Many cases of zygospore formation seen.

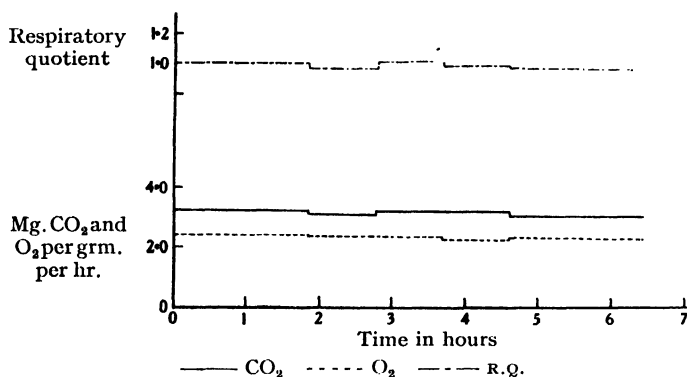


Fig. 7. Respiration of *Sirogonium* without conjugation.

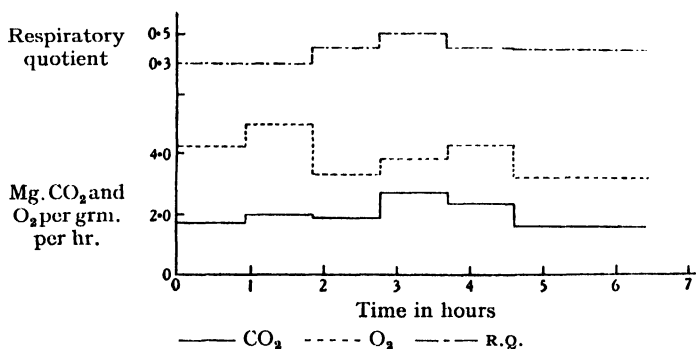


Fig. 8. Respiration of conjugating *Sirogonium*.

Fig. 8 gives the respiration of conjugating *Sirogonium*, in which it should be noted that only a few cells take part in conjugation. The

respiratory intensity is lower than in the previous case, being about 2.0 mg. carbon dioxide and 3.5 mg. oxygen. The R.Q. falls to the neighbourhood of 0.38.

(b) *Oil and starch content in conjugating Spirogyra.*

A search through the available literature showed no record bearing on the physiology of conjugation. Low R.Q. values have, however, been obtained in many plants, especially when fats and oils are utilised for respiration purposes. Many investigators have found a great accumulation of oil in the zygospores of members of the Conjugatae(22). Though it is stated that during germination the oil present in the zygote is reconverted to sugar, the exact time of formation of the oil found in the zygote has not yet been worked out. Fritsch(22), however, reported the presence of oil in the gametes of *Sirogonium* before actual fusion of the gametes.

Therefore, in order to know how far oil was responsible for the peculiar R.Q. obtained, a few chemical and microchemical examinations were made.

For tests of oil(24), osmic acid (1 per cent.) solution and Sudan III (saturated solution in 70 per cent. alcohol) were used. Starch also was simultaneously tested for by chloralhydrate iodine solution.

It was found that the vegetative *Spirogyra* contained abundant starch in the chloroplasts but was devoid of any oily matter. (Osmic acid, however, stained the cells black owing to the presence of a great amount of tannin, the presence of which was also proved by staining with ferric chloride(24). However, when tannins were removed by boiling with water and oil was tested for by osmic acid no colour was developed.)

Filaments undergoing conjugation, examined after staining with iodine, showed the presence of starch in all filaments. But starch was more abundant and much larger blue patches were formed in the cells that had sent out conjugation tubes. Cells not so far advanced and those that failed to form conjugation tubes had smaller and fewer starch grains. Stained with Sudan III, the general cytoplasm of the vegetative cells adjoining the conjugating ones turned slightly pinkish, showing diffused oily matter present in the cells. On the other hand, the cells which had conjugating tubes when stained with Sudan III showed big oil globules arranged in the chloroplasts mixed with hyaline globules which were proved to be starch grains by staining with iodine (Fig. 9). Osmic acid failed to colour these oil globules immediately, but on keeping a deep black colour was obtained.

Estimations of ether-soluble matter in *Spirogyra* confirmed the formation of oil during the maturation stage. The algae were first dried at 95–96° C. for 24 hours. The dried material was well crushed in a mortar and ether was added to it. The solution was filtered off and new ether added and the same operations repeated until no soluble matter was left in the material. The method is rather crude, and does not give the absolute amount of oil in a sample, but serves well to show any change in fat content, provided the amounts of other ether-soluble substances remain unchanged.

The ether-soluble matter thus obtained in normal *Spirogyra* was 3.4 per cent. of the dry weight; and in two samples of conjugating

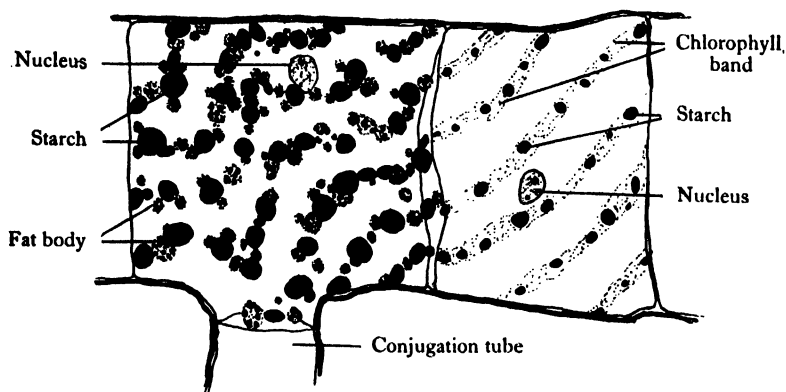


Fig. 9. Part of a conjugating filament of *Spirogyra*.

filaments in which there were no zygospores was 6.1 per cent. in one case, and 5.2 per cent. in the other.

Estimations of reducing sugars were also made both in normal and conjugating filaments by Pavy's solution, but in both cases the amounts were so small that no definite results were obtained.

(c) *Diastatic and lipasatic activity in Spirogyra.*

Both normal and conjugating filaments were now examined for diastatic and lipasatic activity. The filaments were hand pressed to squeeze out extra water and then weighed. Thrice the weight of water was now added and the solution was allowed to stand for a few minutes. Then it was filtered and tested for the enzymes in the following manner.

Diastatic activity was estimated by Wohlgemuth's iodine method (20). Various concentrations of enzyme solutions were taken in uniform test-tubes and 5 c.c. of enzyme solution and 0.5 c.c. toluol

were added to each. The tubes were then well shaken, stoppered and incubated at 40° C. for 20 hours. After the period of incubation, they were immediately cooled and three drops of *N/10* iodine solution added. The tube of highest dilution which developed a blue-red colour was taken to represent the limit of enzyme activity. The results are given in Table I, which were identical in both normal and conjugating filaments.

TABLE I

Enzyme c.c.	5	2	1	0.5	0.3	0.2	0.1
Iodine reaction	Yellow	Yellow	Yellow	Red-yellow	Blue	Blue	Blue

Thus the diastatic activity in both normal and conjugating filaments was $\left(\begin{matrix} 40^{\circ} \text{ C.} \\ D \text{ 20 hours} \end{matrix} \right) 10$.

The lipasatic activity was estimated by Kanitz' method(20). A little phenolphthalein was added to some commercial olive oil which was shaken with a few drops of decinormal sodium hydroxide until a uniform emulsion of slightly pinkish colour was obtained. 10 c.c. of this emulsion were taken in different flasks containing 5 c.c. of enzyme solutions. These were now incubated at 5° C. for 24 hours. Similar flasks were taken in which enzyme solution was added after boiling for about 2 min. After the period of incubation the flasks were taken out and 50 c.c. of 95 per cent. alcohol and 5 c.c. ether were added to each. The mixtures were then titrated against standard decinormal sodium hydroxide. The average of the controls were subtracted from the average of the flasks containing unboiled enzyme and the amount of acid produced calculated.

It was found that the lipase activity of normal *Spirogyra* filaments without any conjugating ones was negligible. But, in conjugating filaments, 5 c.c. of the enzyme solution converted 92.9 mg. oleic acid in 24 hours.

Thus, in the conjugating filaments, the diastatic activity remains equal to that of normal filaments but the lipasatic activity which is absent in normal filaments greatly increases in the conjugation stages.

B. Discussion

The *Spirogyra* filaments require a few days' time to pass through all stages of conjugation, and as will be shown in Section 2 of this paper darkness greatly hinders this process. Hence it is not possible

to estimate the gradual drift of respiratory intensity in conjugating *Spirogyra* in a single experiment.

However, by joining the initial respiratory values obtained in different experiments with *Spirogyra* in various stages of conjugation one can construct a hypothetical curve showing the respiratory intensities at these stages. Fig. 10 has been constructed in the manner mentioned above, showing the initial carbon dioxide output, oxygen intake and the respiratory quotient at these different stages. A fairly good idea of the respiratory mechanism can be obtained from these curves.

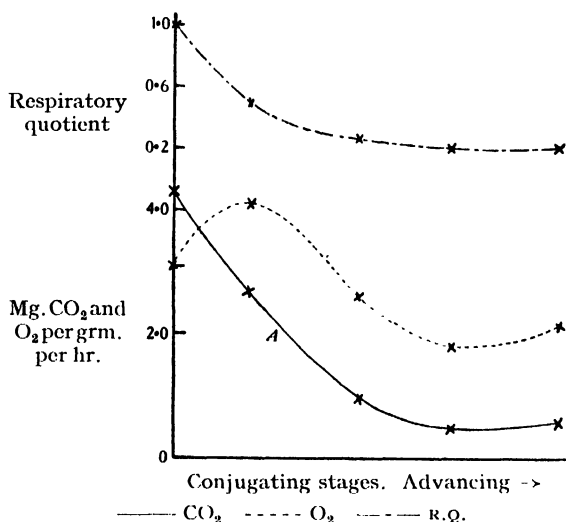


Fig. 10. Respiration of conjugating *Spirogyra* at different stages.

It is now proposed to examine the points of interest arising out of these curves separately.

(a) *Intensity of carbon dioxide emission at different stages of conjugation.*

The unbroken line in Fig. 10 represents the carbon dioxide emission per grm. per hour at different stages of conjugation. It will be noticed that in normal *Spirogyra* the respiratory intensity is comparatively high, being about 4.3 mg. CO₂ per hour. However, as the filaments send out conjugation tubes, a physiological change takes place in the protoplasm and the carbon dioxide emission falls off, the intensity being about 1 mg. CO₂ by the time the conjugation tubes are fully formed. By the next stage, when the chlorophyll bands disorganise and the cell contents begin to pass off, the intensity falls

still lower to the value of 0.5 mg. CO₂, and thereafter remains fairly constant. The lowest value reached is only about 12.5 per cent. of the intensity in normal filaments.

At first glance, this very steep fall in the amount of carbon dioxide given off seems to be very confusing. The protoplasm in these stages is in quite an active state. The movement of the filaments for the side by side arrangement, the formation of the beak, the internal changes in the cell contents and finally the passing out of these contents, all require, we should expect, a great amount of energy. But the carbon dioxide emission is only 12.5 per cent. of that of normal filaments. If the energy release is proportional to the carbon dioxide production, only 12.5 per cent. of the energy released in normal *Spirogyra* is available during the final stages of conjugation.

This seems to be an abnormally low figure in view of the protoplasmic activity. Moreover, the experiments have shown that the lipase activity actually increases and the diastatic activity remains constant in these stages, showing that the enzyme activities of the filaments are in no way deficient.

At this stage, therefore, the only conclusion that can be drawn is that the carbon dioxide emission is not a true measure of the total amount of energy released in the conjugating phase. This point will again be taken up later in this paper.

(b) *The oxygen intake in conjugating Spirogyra.*

The dotted line in Fig. 10 represents the oxygen intake in *Spirogyra* at different stages of conjugation. In normal filaments it is about 3 mg. per hour. But as the conjugating tubes appear this value first mounts to 4 mg. per hour and then it begins to fall until the zygospore formation stage is reached, when it remains at about 2 mg. per hour. The uneven nature of the curve is fundamentally different from the carbon dioxide output curve which shows a continuous fall from the beginning. It is evident that the relation between the oxygen intake and the carbon dioxide output is not constant as in normal plants.

The higher amount of oxygen intake and the smaller fall in this value may be due to the fact that the oxygen taken in, during the conjugation stages, is used in reactions other than that of oxidising sugars completely into carbon dioxide and water. This will be further clarified under the next heading.

(c) *The respiratory quotient in conjugating Spirogyra.*

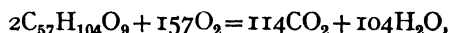
The dot and dash line in Fig. 10 shows that like the carbon dioxide output the respiratory quotient values also fall off with advancing stages of conjugation. This, together with the lower carbon dioxide emission, strongly suggests that there is a great change in the metabolic activity in conjugating *Spirogyra*.

Respiratory quotient values lower than unity have been obtained in many plants, specially in those which use fats and oils as a substrate for respiration. Some results of this kind obtained by different authors are given below.

Organisms	R.Q.	Authors
1. <i>Phycomyces</i> grown on ground linseed medium	0.65-0.75	De Boer (3)
2. Lupin seeds	0.54	Bonnier and Mangin (1)
3. Flax	0.3-0.64	Bonnier and Mangin (1)
4. Linseed	0.64	Gerber (5)
5. White mustard seed placed in different concentrations of CO ₂	0.45-0.82	Kidd (10)

Very recently, Stiles and Leach (19) have conducted estimations of R.Q. in different seeds with their improved katharometer. They also find that those containing fat reserves have very low R.Q. values.

Such low values are obtained as a consequence of the low oxygen content in fats, which require a great amount of oxygen for complete oxidation. For example, when triglyceride of ricinoleic acid (the chemical nature of the fat found in *Spirogyra* being unknown, this fat has been used for all considerations and calculations in this paper) is oxidised according to the following equation:



the R.Q. would be only $114/157 = 0.73$.

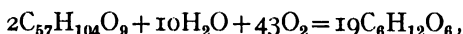
The presence of fat at the conjugation stage in *Spirogyra* and *Sirogonium* and the considerably greater lipasatic activity support the view that the low R.Q. obtained may be due to the oxidation of this material.

But the R.Q. should not, in ordinary cases, be much lower than 0.7 when the fat is completely oxidised to carbon dioxide and water, whatever fat is used for the purpose. Therefore, such extreme values as 0.2 cannot be explained by the simple oxidation of fats to carbon dioxide and water.

Regarding the chemistry of oxidation of fats in plants nothing as yet is definitely known. It is, however, a well-established fact

that, in seeds, the carbohydrates begin to accumulate as the fats disappear. Thus carbohydrates are formed by conversion of fats. This was observed by de Saussure as far back as 1842 and has since then been confirmed by various authors. This partial oxidation of the fats requires a great amount of free oxygen.

Thus the very low R.Q. values may be due to the conversion of fats to carbohydrates. Stiles and Leach⁽¹⁹⁾ have shown that if only half of a given amount of the triglyceride of ricinoleic acid is oxidised to carbon dioxide and water and the other half converted to sugar according to the following theoretical equation:

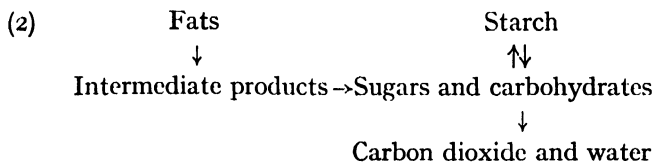
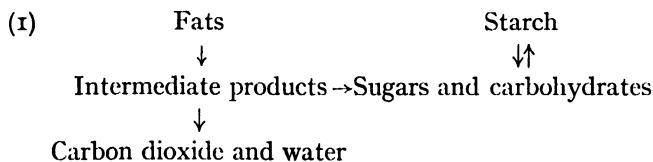


the R.Q. would be only $114/157 = 0.57$. Similarly, if only a third part is respired while the rest is converted to sugar the R.Q. would fall to 0.47.

Thus it may be concluded that the very low R.Q. in conjugating filaments is due to a very rapid conversion of oils into carbohydrates, by which process a great amount of oxygen is fixed without any liberation of carbon dioxide.

(d) *Substrate used for respiration in conjugating Spirogyra.*

That in normal *Spirogyra* the substrate used for respiration is sugar as in all other plants is shown by the fact that the R.Q. in these filaments is unity. However, in the conjugating ones it is found that oils take a great part in the metabolism. It has also been pointed out that the oil is not completely oxidised to carbon dioxide and water, but a great amount of it is converted to sugar. These changes can take place in two different ways:



According to the first scheme the fats are directly oxidised to carbon dioxide and water without being converted to sugars. Thus the substrate for respiration becomes fat and not sugar, though, even

in this case, a considerable amount of fat is converted to sugar. The point, therefore, at once arises why the normal respiratory process should stop and instead of carbohydrates which are abundant, the oils should begin to be utilised for the purpose.

As has been pointed out before, in no stage of conjugation are the filaments very deficient in starch content. The diastatic activity also remains constant throughout, showing that starch \rightleftharpoons sugar reaction is not greatly altered in conjugating filaments. Therefore in view of the abundance of starch and simple carbohydrates it does not seem probable that the oxidation of sugar to carbon dioxide is greatly affected by light, which is a finding contrary to the first scheme.

According to the second scheme, however, the fats are first oxidised to carbohydrates, which are themselves then further oxidised to carbon dioxide and water. Thus the normal respiratory process continues, and only a side reaction of conversion of fats to carbohydrates is added, which necessitates a high intake of oxygen thereby greatly lowering the R.Q. The greater the magnitude of the side reaction, the lower becomes the R.Q.

Though both the schemes are possible, the second one seems to be more probable in view of the above considerations.

(e) *Fate of oxygen taken in.*

From the above it is seen that the oxygen taken in is used for two separate reactions:

- (1) For the oxidation of sugars to carbon dioxide and water, and
- (2) For conversion of oil to sugar and carbohydrates.

The amount of oxygen used for the first reaction can be calculated from the carbon dioxide emission of the algae on the basis that the ratio between the volumes of the two gases is unity. Then subtracting this amount from the total amount taken in, one can also have a measure of the amount used in the second reaction. Thus the total quantity of oxygen taken in can be broken up into two component parts. Table II gives these calculated values.

TABLE II

Experiment represented by Fig. no.	Carbon dioxide output mg.	Amount of oxygen taken in mg.	Oxygen used for oxidation of sugar mg.	Oxygen used for conversion of oil mg.
2	4.31	3.12	3.12	—
3	2.72	4.09	1.98	2.11
4	0.97	2.57	0.71	1.86
5	0.44	1.77	0.32	1.45
6	0.60	2.1	0.44	1.66

Fig. 11 gives the amounts of oxygen used for oxidation of sugars (A) and for conversion of oil (B) separately in a graphical form. It will be seen from this that while A necessarily corresponds with the carbon dioxide curve of Fig. 10, B is much smoother than the oxygen curve of Fig. 10. The amount of oxygen used for the conversion of oil keeps fairly constant, thereby showing that the reaction goes on practically at constant rate. Thus the uneven nature of the total

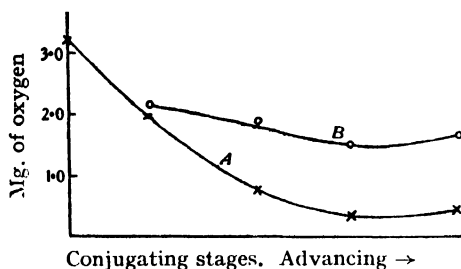


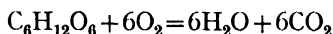
Fig. 11. Fate of oxygen taken in by conjugating *Spirogyra*. A. Oxygen used for oxidising sugar. B. Oxygen used for conversion of fat to carbohydrates.

quantity of oxygen taken in is only due to the summing up of the amounts used for two separate reactions and not due to any great uneven variation in any of these.

(f) *True index of energy released in conjugating Spirogyra.*

Ordinarily, the amount of carbon dioxide emission is taken to be the measure of respiratory intensity. However, respiration in its true sense includes all those reactions which release energy for the use of the organisms. As previously shown oils are oxidised to carbohydrates in conjugating *Spirogyra*. Oils are of higher energy content than the carbohydrates and therefore in this reaction a great amount of energy is liberated. The following considerations are therefore necessary in order to understand the true respiratory intensity in *Spirogyra*.

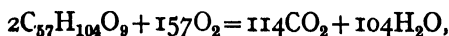
When a gram of glucose is oxidised to carbon dioxide and water 3.76 K. of heat is given out; therefore, in the following reaction,



per gram of oxygen used $3.76 \times 180/192 = 3.525$ K. of heat will be given out.

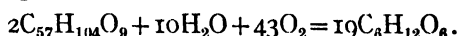
Similarly, the energy given out when the fats are oxidised to carbohydrates can be calculated.

Every gram of fat when oxidised to carbon dioxide and water gives out 9.1 K. of heat;



i.e. 1864 grm. of fat when oxidised will give out 9.1×1864 K. of heat.

But in *Spirogyra* the reaction stops at the carbohydrate stage; thus part of the energy that would have been free is utilised in the formation of sugars,



Thus 1864 grm. of fat gives 19×180 grm. of sugar, which has an energy content of $19 \times 180 \times 3.76$ K. of heat. Therefore in the above reaction 1864 grm. of fat and 180 grm. of water combining with 1376 grm. of oxygen gives out 9.1×1864 K. + 587 K. (energy content of water) — $19 \times 180 \times 3.76$ K. = 4690.2 K. of heat. Thus 1 grm. of oxygen utilised in the above reaction gives $4690/1376 = 3.4$ K. of heat.

Thus from the amount of oxygen used in the different reactions of Table II the actual amount of energy released can be calculated. Table III gives these values.

TABLE III

Experiment represented by Fig. no.	Oxygen used for oxidation of sugars mg.	Oxygen used for conversion of fat (mg.)	Energy from oxidation of sugars	Energy from fats	Total amount of energy
2	3.12	—	10.98 K.	—	10.98 K.
3	1.98	2.11	6.97 K.	7.17 K.	14.14 K.
4	0.71	1.86	2.50 K.	6.32 K.	8.82 K.
5	0.32	1.45	1.13 K.	4.93 K.	6.06 K.
6	0.44	1.66	1.55 K.	5.64 K.	7.19 K.

The total energy released in different stages is represented in Fig. 12. The curve at first shows a rise, then a fall, finally ending in a level

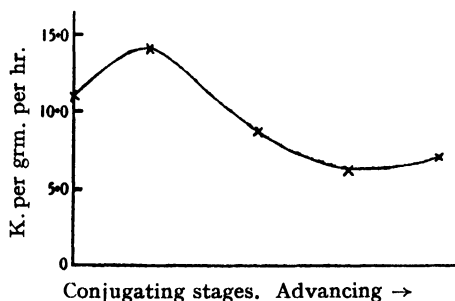


Fig. 12. Energy released in conjugating *Spirogyra*.

course. Therefore the conclusion arrived at is that the activity of conjugating *Spirogyra* increases in the first stage while the beak is

being formed. This is followed in the second by the gradual slowing down of the activity while the cell contents begin to disorganise. While in the third stage, when the protoplasm of one of the gametes begins to pass off to the other, the activity is about the lowest, being roughly half the intensity of that of normal filaments. This finding is quite in accordance with what is expected; for in the first stage, owing to the movement of the filaments for side-by-side arrangement and the formation of the beak, the protoplasm is in an intensely active state; while in the third stage, the protoplasm gradually stops all normal functions and is in a low state of activity.

A comparison of the energy release curve and the carbon dioxide curve would bring out the great divergence between the two. Thus it is evident that, in conjugating *Spirogyra*, the carbon dioxide emission curve cannot be employed as an index of the metabolic activity. While, on the other hand, the oxygen intake is to some extent proportional to the energy release and therefore its study can to some extent serve the purpose, though for a full investigation an analysis of the actual amount of heat developed would be necessary.

(g) *Oil reserve in Spirogyra.*

The foregoing discussions therefore lead us to conclude that during the process of conjugation, part of the oil present is oxidised to sugar. But as has been pointed out before, the staining tests and the estimations of the ether-soluble matter have shown that actually fats begin to accumulate only when conjugation tubes are being protruded.

At this stage, the only explanation possible is that oil begins to form just before conjugation starts and its presence is overlooked at the time. This previously accumulated oily matter is oxidised again in the conjugation stages. But such reactions are highly improbable in view of the fact that a great amount of oil is found in the zygote. Moreover, in that case, there should be a certain stage when owing to the formation of oil the R.Q. would be greater than unity. Though several experiments were conducted to find the stage out, in no experiment was the R.Q. obtained greater than unity; it was either unity (in normal filaments) or less than unity (in filaments in any stage of conjugation).

It was later found that the reversibility of the reactions was greatly influenced by light; and the darkness inevitable for respiration experiments was responsible for the conversion of oil to sugar. This point will be taken up further when dealing with the light experiments.

SECTION 2. EFFECT OF LIGHT ON THE PHYSIOLOGY OF
CONJUGATION

A. *Experimental results*

The following four experiments were now performed with light. The method of experimentation has been dealt with in the introductory part of this paper.

In the first three experiments normal *Spirogyra* filaments without any lateral attachments were employed, while in the last experiment conjugating filaments were taken.

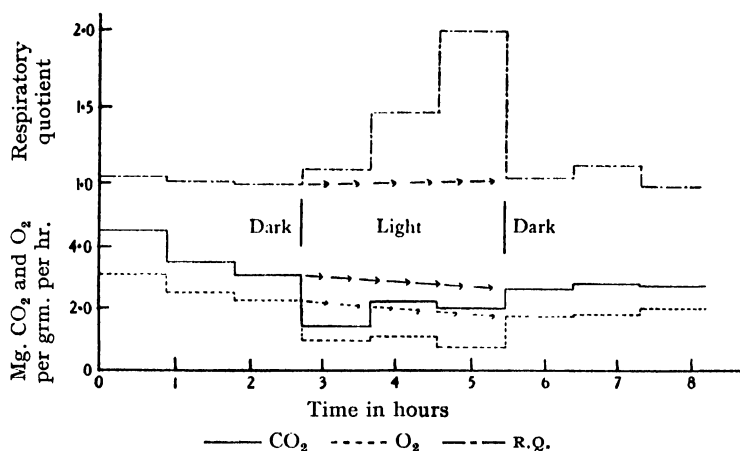


Fig. 13. Effect of light on respiration of *Spirogyra*.

In the first, Fig. 13, the algae were allowed to respire for three periods in darkness and then the light was switched on for another three periods, after which the experiment was again continued for three periods in the dark. The respiration originally started high, 4.45 mg. carbon dioxide and 3.05 mg. oxygen, and then like the typical curve fell steadily up to the end of the third period. Light was now given and the respiration fell off suddenly and remained low, until the light was put out when it again rose. The main point of interest is the R.Q. Originally it was near to unity, but as soon as light was given it rapidly rose so that in the third period the ratio was 2. When again in darkness it immediately fell to unity.

In the next experiment, Fig. 14, a longer exposure to light was given in order to ascertain its influence on the R.Q. The algae were placed in the dark for two periods, then for six periods in light and finally for two periods in the dark again. The graphs obtained are

fundamentally the same as in the last experiment, and the R.Q. shows a maximum of about 2.

In the third experiment, instead of employing artificial light, the

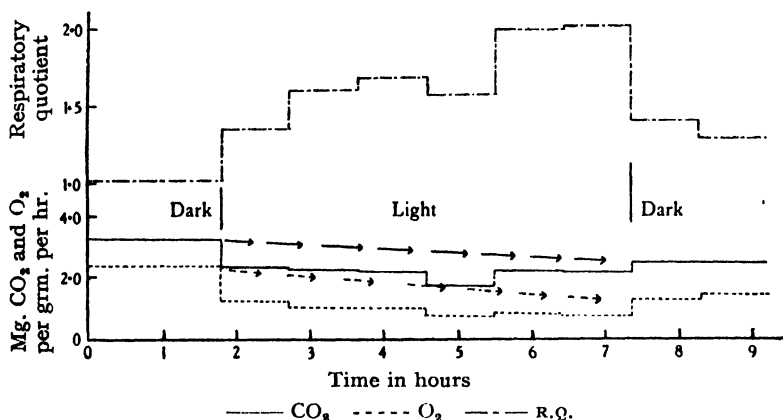


Fig. 14. Effect of light on respiration of *Spirogyra*.

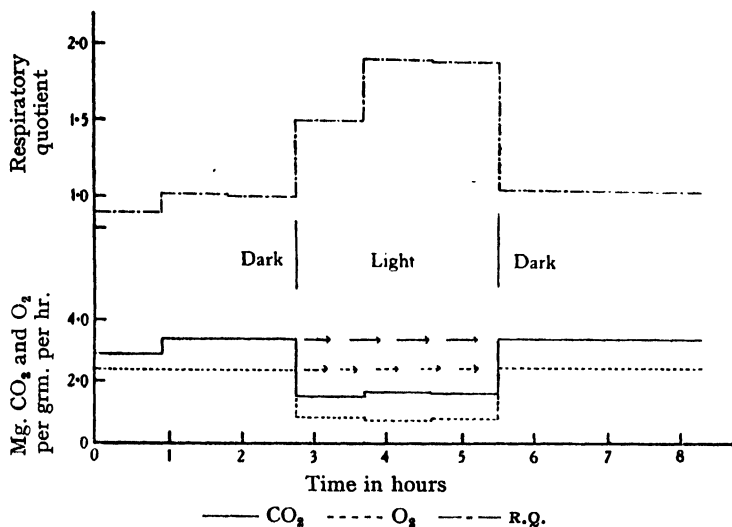


Fig. 15. Effect of sunlight on respiration of *Spirogyra*.

apparatus was taken out in the open and the algae were exposed to sunlight (Fig. 15). The sky was clear and the algae received full midday light. Here also the graph obtained was similar to the previous ones, thereby showing that both artificial light and sunlight have a similar effect on the algae.

In the last experiment, Fig. 16, conjugating filaments were employed instead of normal ones. Almost all the filaments showed formation of tubes, but no passage of protoplasm was seen. Artificial light was employed in this experiment. The carbon dioxide emission started rather low as is characteristic of conjugating filaments. When exposed to light it fell further and kept low throughout the period in light, and again rose when taken back to darkness. The R.Q. in light did not rise to the high values attained in the other experiments, but at first showed a slight fall, and then a rise which kept constant even when the alga was taken back into the dark.

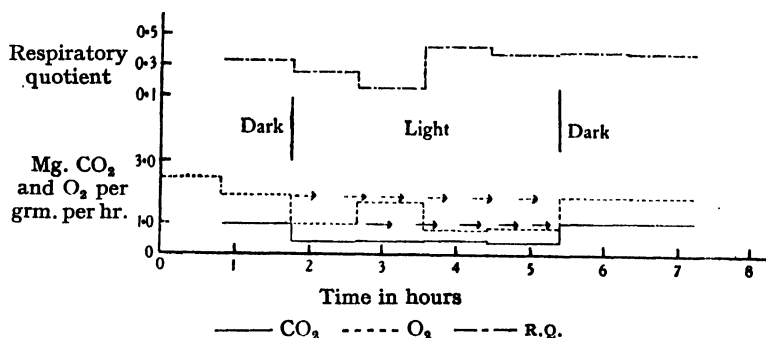


Fig. 16. Effect of light on respiration of conjugating *Spirogyra*.

B. Discussion

(a) Effect of light on respiration in *Spirogyra*.

All the experiments with light show that the carbon dioxide emission and oxygen intake fall greatly on exposure to light. This is surely due to part of the carbon dioxide given out by respiration being assimilated on exposure to light. The lower intake of oxygen is also due to the evolution of the gas in assimilation. It is to be noted here that no extra amount of carbon dioxide was allowed to the plants, and therefore the only source of it was the amount given in respiration. However, before all the gas evolved in respiration is assimilated part of it escapes out into the surrounding medium, and therefore a fair amount of carbon dioxide is detected even on exposure to light.

Regarding the influence of light on respiration, there exists no difference of opinion as to the photosynthetic part of it. Since Borodin(2) showed that after an exposure to light the leafy shoots undergo more vigorous respiration, the indirect influence of light on

plants having chlorophyll has been confirmed by various authors. This is due at any rate in part to the high concentration of sugars formed by photosynthesis.

Regarding the indirect effects of light apart from the photosynthetic one, the results so far obtained are contradictory and probably inconclusive. Thus the experiments of Bonnier and Mangin (1), Elfving (4), and Lowschin (11) showed a somewhat retarding effect of light on respiration. It is to be noted that these experiments were performed with parts of plants devoid of chlorophyll. Maximov (12) found little or no effect of light on respiration.

On the other hand, Meyer and Deleano (13) noted a daily periodicity in respiration under natural conditions. Spoehr (18), from his experiments, came to the conclusion that during the day the respiration of plants increases on account of ionisation of the air brought about by the sun. He suggested that owing to ionisation of the oxygen, autoxidation is instigated in the protoplasm. Middleton (14), with barley seedlings, and Whimster (23), with leaves of *Pelargonium zonale*, have found a great increase of carbon dioxide production in presence of ionised air, the effect being greater in green leaves.

Very recently Ranjan (17), from his experiments with *Croton* leaves, concluded that apart from the photosynthetic influence, light greatly enhances the respiration of leaves. In a paper under publication, he has shown that the enhancing effect is also true of *Pistia* shoots. He, however, found no such effect on *Pistia* roots. This he believes to be due to the lack of anthocyanins in the roots, which are thus unable to absorb the light rays.

Very recently Parija and Saran (16) have found that light accelerates the respiration of *Aralia* sp.

The respiration curves of *Spirogyra* in light do not warrant the conclusion of any marked effect of light on the carbon dioxide emission. When the plants were put back in the dark after an exposure to light, the respiration rose to a level which coincided with the probable intensity of normal *Spirogyra* left in darkness continuously (cf. Fig. 2).

According to Ranjan all plants in which respiration is enhanced by light show a gradual fall in respiration when put in the dark. This feature is entirely absent in the experiments with *Spirogyra*. It is therefore to be concluded that there is no direct effect of light on the respiration of these plants.

Then by joining the respiration values before and after the exposure to light, the probable respiration curve in *Spirogyra* during the period in light can be obtained. The arrow-head lines in Figs. 13-

16 thus represent the true respiration of the filaments during the period. It is to be seen that the true R.Q. due to respiration only remains constant throughout. The rise in the observed R.Q. is to be attributed to some indirect effect of light, the consideration of which will be taken up later.

(b) *Assimilation of Spirogyra in absence of external supply of carbon dioxide.*

The arrow-head lines in Figs. 13-16 represent the measure of respiration in light. From these and the actual amounts of carbon dioxide given out and oxygen taken in by the plants, the amounts of carbon dioxide used and oxygen given out in assimilation can be ascertained. Table IV shows these calculated amounts. The assimilatory quotient (volume of carbon dioxide/volume of oxygen) is also shown.

TABLE IV

1	2	3	4	5	6	7	8
Period in light	Actual amount of carbon dioxide given out mg.	Hypothetical respiration mg.	Carbon dioxide assimilated mg.	Actual amount of oxygen mg.	Oxygen taken in for respiration mg.	Oxygen assimilated mg.	Assimilatory quotient
<i>Experiment represented by Fig. 13</i>							
1	1.42	3.0	1.58	0.93	2.1	1.16	1.02
2	2.19	2.8	0.61	1.11	2.0	0.89	2.0
3	2.04	2.7	0.66	0.75	1.9	1.15	2.4
<i>Experiment represented by Fig. 14</i>							
1	3.1	3.1	0.80	1.24	2.2	0.96	1.65
2	2.22	2.90	0.68	1.01	2.0	0.99	2.0
3	2.18	2.8	0.62	0.94	1.80	0.86	1.91
4	1.69	2.7	1.01	0.78	1.6	0.82	1.26
5	2.20	2.6	0.4	0.80	1.6	0.8	2.75
6	2.16	2.5	0.34	0.76	1.5	0.74	2.99
<i>Experiment represented by Fig. 15</i>							
1	1.66	3.4	1.74	0.76	2.4	1.64	1.3
2	1.7	3.4	1.7	0.66	2.4	1.74	1.39
3	1.66	3.4	1.74	0.64	2.4	1.74	1.38
<i>Experiment represented by Fig. 16</i>							
1	0.35	1.0	0.65	0.91	1.84	0.93	1.97
2	0.45	1.0	0.55	1.7	1.85	0.15	0.38
3	0.47	1.0	0.53	0.79	1.86	1.06	2.72
4	0.45	1.0	0.55	0.82	1.87	1.03	2.59

Column 4 of the table gives the amounts of carbon dioxide actually assimilated during the periods in light. This amount is very much less than what is expected. Under favourable conditions assimilation is several times greater than respiration in the same period. As has been said before the cause of this lies in the fact that no external

carbon dioxide was allowed to the plants, and thus only the amount given out in respiration could serve as a source of carbon dioxide for assimilation. The low intensity of assimilation is therefore only due to shortage of carbon dioxide available to the plants.

However, in spite of the fact that the plants had sufficient light, only a portion of the carbon dioxide respired was assimilated; the rest diffused out into the surrounding medium. It is evident that these plants cannot assimilate the carbon dioxide given off in respiration quite so quickly as the gas diffuses off into the surrounding liquid. The relation between the two alternating fates of the carbon dioxide given out in respiration (diffusion and utilisation by photosynthesis) is not constant and seems to be rather obscure, probably due to interaction of some other factors.

(c) *Assimilatory quotient and influence of light on oil formation in Spirogyra.*

Fig. 17 gives the assimilatory quotient values from Table IV obtained by calculations in a graphical form. It is considerably

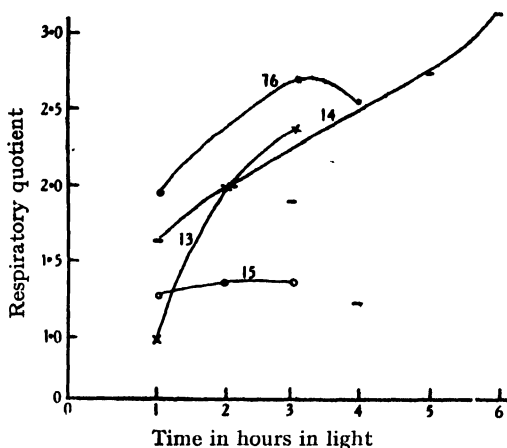


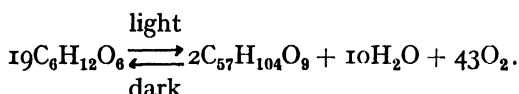
Fig. 17. Assimilatory quotient in *Spirogyra* in water free from carbon dioxide.

greater than the expected value of unity. Such a high oxygen emission immediately suggests the probability of its production in some process other than that of assimilation of carbon dioxide and water to sugar.

The appearance of oil and the failure to obtain R.Q. values greater than unity at any stage in the respiration experiments can now be

correlated with the high evolution of oxygen in light. It is quite possible that the extra amount of oxygen given out in light experiments is due to its evolution in formation of oil from sugar. The oil found in *Spirogyra* thus begins to form at a stage previous to conjugation, and, as would be evident from the assimilatory quotient of conjugating *Spirogyra*, the process continues vigorously till the end.

This reaction, however, takes place only in light. Thus in the dark experiments the R.Q. was never greater than unity. In absence of light, the reverse process of oxidation of fats begins and consequently a great amount of oxygen is taken in:



Ivanow (9) has shown that lipase can either hydrolyse a fat or may synthesise it from fatty acid and glycerol. He found that if a glycerol extract of seed be mixed with oleic acid, fat is synthesised, while it is again split up on diluting with water.

No influence of light on the reversibility of lipasatic action is as yet known, but the peculiar oil metabolism strongly suggests such an influence.

It is to be noted here that in no light experiment was the normal assimilatory quotient of unity obtained. There can, however, be no doubt that in young vegetative filaments a value very near unity would be obtained. In the season when these experiments were carried out no young and rapidly growing filaments could be procured to verify this assumption. But the rather low assimilatory quotient values in the experiment with sunlight show such a possibility. Probably in this case the condition of the filaments was not so advanced as in the other cases. Another fact to be noted in this connection is that the unfavourable laboratory conditions, the high temperature and the starting of experiments in pure water hasten the conjugation stages, and the normal conditions are difficult to obtain.

(d) *Influence of light on conjugation in Spirogyra.*

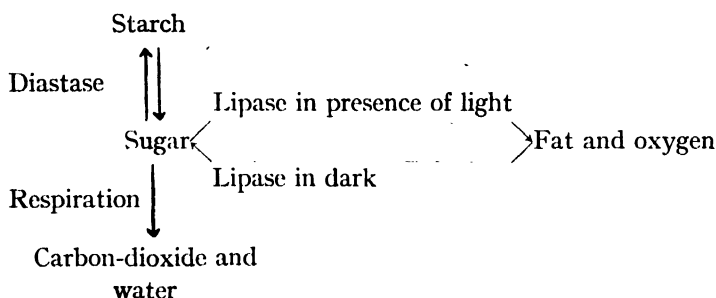
The importance of light on the formation of oil in *Spirogyra* has been shown under the last head, and now it is important to know whether light has any influence on the process of conjugation itself. The following experiment was performed in order to have an idea of this problem.

Several samples of normal *Spirogyra* were taken in different dishes containing pond water, distilled water and 0.05 per cent. sodium bicarbonate solution. One set of these was kept in a corner in the laboratory in diffused light, while the other was in the open in sunlight. Though the filaments in most of the cases in light showed beak formation within 2-7 days, in no case of the other set was any stage of conjugation seen. After about a week and a half these filaments died. Thus even in unfavourable circumstances when the vegetative filaments cannot thrive the sexual stages are inhibited by weak light.

Thus strong light is essential for conjugation in *Spirogyra*. Recently Garnar and Allard (6), Hendricks and Harvey (8), Wann (21) and others have found that light has a marked influence on the reproduction and blooming of various plants. The cause of this has not yet been definitely ascertained, and the various views seem to be speculative. The experiments with *Spirogyra* do not give any clue to this problem, but it is seen that light influences the formation of oil, which is intimately connected with the process of conjugation.

(e) *Economics of oil metabolism in Spirogyra.*

As a result of the discussions given above the following scheme is suggested to represent the reactions in conjugating *Spirogyra*:



As the conjugation stages advance, more and more of the reserve starch gets converted to fat; so that in the final stage when the zygospore is fully formed it contains only fats. The fat in the zygospore is used as a reserve food during germination, when it is again converted to carbohydrates. In higher plants also many seeds contain oily reserve matters. The chief advantages therefrom are that only a small space is required for the storage (for the carbohydrates of the same energy content have a greater bulk), and that the storage of oil minimises the danger of draught.

The conversion of food matters to oil is thus of great importance

to the plants. The question now arises as to the special advantage of the reversibility of the reaction in the dark in *Spirogyra*.

The plants in these stages, as has been shown before, require a considerable amount of free energy. At the same time, however, they also require a good accumulation of food reserve to be used during the germination of the zygospores. Conjugation and other sexual processes in the algae are only adaptations to tide over some unfavourable conditions when vegetative growth becomes impossible. Shortage of food material is very often the cause of the beginning of the sexual processes. Thus in these stages the filaments have great difficulty in supplying enough food matter for respiration purposes without greatly minimising the food reserve in zygospores.

The peculiar oil metabolism in *Spirogyra* is the instrument by which the loss of food matter in respiration during darkness is minimised. During darkness, instead of deriving energy from the oxidation of sugars and thereby releasing the carbon dioxide thus evolved, part of the energy required is obtained by conversion of oil to sugar. In light the sugar so formed and the reserve carbohydrates are again converted to oil, the light energy being used for the purpose. Thus without any great loss of carbohydrate food an adequate supply of energy is achieved.

SUMMARY

A simple apparatus, being an improvement on the adaptation of Winkler's method by Osterhout and Haas, for estimating the respiratory and photosynthetic activities in water organisms has been described.

It has been found that the evolution of carbon dioxide in normal *Spirogyra* and *Sirogonium* is comparatively high and the respiratory quotient equal to unity, but as the sexual stages progress, the carbon dioxide emission and the respiratory quotient gradually fall to very low values. The intake of oxygen, however, shows at first a rise and then a lesser fall.

It has also been found that though purely vegetative filaments do not contain any oily matter, a great number of oil globules appear in the filaments undergoing conjugation. Starch is abundant at all times except in the very latest stages of zygospore formation, when it is completely converted to fats and oils.

It has been suggested that the very low respiratory quotient in conjugating *Spirogyra* and *Sirogonium* is due to a very rapid oxida-

tion of fats and oils to carbohydrates. This necessitates the fixing of a great amount of oxygen without any liberation of carbon dioxide.

As the oxidation of oils to carbohydrates liberates a considerable amount of free energy, the evolution of carbon dioxide in conjugating *Spirogyra* cannot be used as an index of the metabolic activity of the organisms. On the other hand, this purpose can fairly be served by the intake of oxygen, for the energy liberated per gram of oxygen used for oxidation of sugars is approximately equal to the energy released per gram of oxygen used for oxidation of fats to carbohydrates.

The metabolic activity of the conjugating *Spirogyra* increases, as evidenced by the oxygen intake, in the first stages of conjugation, and then it falls to about half the value of normal *Spirogyra*.

Light is of great importance to conjugating *Spirogyra*. It is only in light that the conversion of carbohydrates to fats and oils is possible, the light energy being used for the purpose. In the absence of light, the reverse process of oxidation of oils to carbohydrates takes place. Conjugation fails to take place in the absence of light.

The conversion of carbohydrates to oils in light and the reverse process in dark are of great economic importance to the organisms. This process enables the filaments to prevent the loss of carbon while respiring in the dark.

The writer wishes to express grateful appreciation to Dr S. Ranjan for helpful suggestions and criticisms and for his interest in the progress of this study.

REFERENCES

- (1) BONNIER, G. and MANGIN, L. Recherches sur la respiration des plantes sans chlorophylle. *Ann. Sci. nat. Sér. 6*, **18**. 1884.
- (2) BORODIN, J. P. *Physiologische Untersuchungen ueber die Atmung beblätterter Sprossen*. St Petersburg. 1876.
- (3) DE BOER, S. R. Respiration of *Phycomyces*. *Rec. trav. bot. Néerlandais*, **25**. 1928.
- (4) ELFFVING. *Studien über die Einwirkung des Lichtes auf die Pflanze*. 1890.
- (5) GERBER, C. Étude comparée de la respiration des graines oléagineuses pendant leur développement et pendant leur germination. *Actes du Congrès Internat. de Botanique, Paris*. 1900.
- (6) GARNAR, W. W. and ALLARD, H. A. Effect of relative length of day and night and other factors of environment on growth and reproduction in plants. *Journ. Agric. Res.* **18**. 1920.
- (7) HAAS, P. and HILL, T. G. *Chemistry of Plant Products*. London. 1929.
- (8) HENDRICKS, E. and HARVEY, R. B. Growth of plants in artificial light. II. *Bot. Gaz.* **77**. 1924.
- (9) IVANOW, S. Über Ölsynthese unter Vermittlung der pflanzlichen Lipase. *Ber. deut. bot. Ges.* **29**. 1911.

Studies on the Respiration of Conjugating Spirogyra 273

- (10) KIDD, F. The controlling influence of Carbon Dioxide. III. *Proc. Roy. Soc. B*, **39**. 1915.
- (11) LOWSCHIN, A. Zur Frage über den Einfluss des Lichtes auf die Atmung der niederen Pilze. *Beih. Bot. Centralblatt*, **23**. 1908.
- (12) MAXIMOV, N. Ueber den Einfluss des Lichtes auf Atmung der niederen Pilze. *Centralblatt für Bakt.* **9**. 1902.
- (13) MEYER, A. and DELEANO, N. T. Die periodischen Tag- und Nachtschwankungen der Atmungsgrösse im Dunkeln befindlicher Laubblätter und deren vermutliche Beziehung zur Kohlensäureassimilation. *Zeitschr. Bot.* **3**. 1911; **5**. 1913.
- (14) MIDDLETON, N. I. The effect of ionised air on the rate of respiration of barley seedlings. *Ann. Bot.* **41**. 1927.
- (15) OSTERHOUT, W. J. V. and HAAS, A. R. C. An adaptation of Winkler's method to biological work. *Journ. Biol. Chem.* **22**. 1917.
- (16) PARIJA, P. and SARAN, A. B. The effect of light on the respiration of starved leaves. *Ann. Bot.* **48**. 1934.
- (17) RANJAN, S. *Recherches sur la respiration des végétaux*. 1932.
- (18) SPOEHR, H. A. Variations in respiratory activity in relation to sunlight. *Bot. Gaz.* **59**. 1915.
- (19) STILES, W. and LEACH, W. Researches on plant respiration. II. Variations in the respiratory quotient during germination of seeds with different food reserves. *Proc. Roy. Soc. B*, **113**. 1933.
- (20) WAKSMAN, S. A. and DAVISON, W. C. *Enzymes*. London. 1926.
- (21) WANN, F. B. Some of the factors involved in the sexual reproduction of *Marchantia polymorpha*. *Amer. Journ. Bot.* **12**. 1925.
- (22) WEST, G. S. and FRITSCH, F. E. *British Freshwater Algae*. Cambridge. 1927.
- (23) WHIMSTER, K. The effect of ionised air on the assimilation and respiration of green leaves. *Ann. Bot.* **41**. 1927.
- (24) ZIMMERMANN, A. *Botanical microtechnique*. English trans. by Humphrey. 1926.

THE SPORE DISCHARGE MECHANISM IN *BASIDIOBOLUS RANARUM*

By C. T. INGOLD

Department of Botany, University of Reading

(With 2 figures in the text)

THE life history of *Basidiobolus ranarum* has been considered in detail by Eidam⁽¹⁾ in 1886 and by Levisohn⁽²⁾ in 1927. Eidam describes violent spore discharge involving the projection of the conidium together with the "basidium," the two separating in mid-air. The mechanism of the discharge does not, however, appear to have been appreciated. It is proposed in this note to describe the rocket mechanism which is apparently responsible for the propulsion of the conidium to a distance of 1-2 cm. This was discovered while examining material of *Basidiobolus* in order to compare it with *Pilobolus*, a fungus which, superficially, it resembles very closely.

Basidiobolus ranarum in the conidial condition can usually be obtained by collecting the excrement of frogs and keeping it on moist filter paper in a Petri dish.

In spite of the striking general resemblance between the mature sporangiophore of *Basidiobolus* and *Pilobolus* the method of discharge in these two fungi is very different. In *Pilobolus* the turgid subsporangial bulb ruptures along a definite line of weakness just below the junction between the sporangium and the columella. The stretched elastic wall of the sporangiophore *below* this dehiscence line immediately contracts squirting out from the subsporangial bulb a drop of sap which carries the sporangium with it. Up to a point the arrangement in *Basidiobolus* is similar. In the subconidial bulb there is a definite line of weakness where dehiscence takes place, but this line of weakness occurs towards the base of the subconidial bulb and not towards the apex as in *Pilobolus*. Further, the elastic region of the wall is *above* this line of dehiscence, not below it.

Mature conidiophores were observed on the excrement of frogs under the low power of the microscope and discharge was watched. As is to be expected discharge takes place so rapidly that no stages in the process can be observed. At one moment the conidiophore appears straight and turgid, the next nothing is to be seen either of

the conidium and its "basidium" or of the remainder of the conidiophore. The appearance of the conidiophore just before discharge is illustrated in Fig. 1 A. The incipient line of dehiscence can be seen, due to the different reflecting powers of the "basidium" and the lower region of the conidiophore and also on account of the somewhat thicker wall of the "basidium." The thicker nature of this wall is due to the fact that it is double in contrast to the single wall composing the remainder of the conidiophore. The discharged conidium is thrown to a horizontal distance of 1-2 cm. The "basidium" is usually thrown about 0.5 cm.

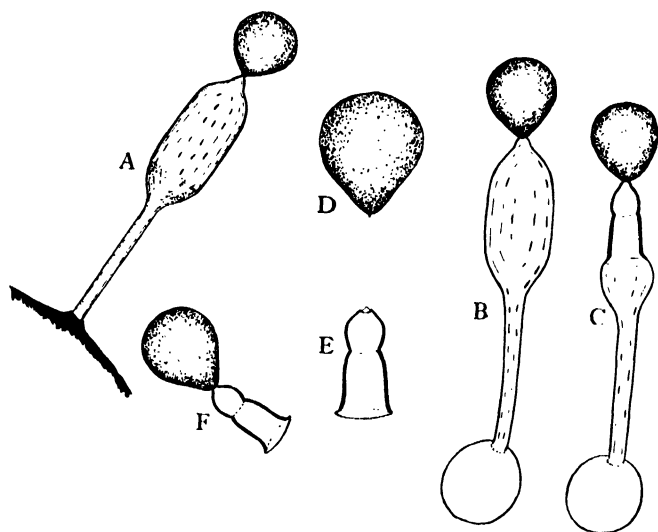


Fig. 1. *Basidiobolus ranarum*. A, mature conidiophore growing on natural substratum; B, living conidiophore mounted in water; C, the same after the addition of a trace of iodine; D, a discharged conidium; E, a discharged "basidium"; F, a conidium and "basidium" which have failed to separate in mid-air. A, B and C $\times 380$; D, E and F $\times 480$.

Fig. 1 B shows a mature conidiophore, derived directly from the germination of a conidium, mounted in a drop of water. Fig. 1 C shows the same conidiophore after being killed by the addition of a trace of iodine. It is to be noted that it is the "basidium" which is the elastic region of the conidiophore wall; the lower region of the conidiophore undergoes no shrinkage on death. This gives the clue to the probable mechanism of discharge. The pressure in the conidiophore becomes so great that rupture occurs along the line of dehiscence below the "basidium." Then the elastic wall of the "basidium" suddenly

contracts; it squirts sap backwards, flies off on the recoil and carries the conidium away with it. The mechanism of discharge resembles that of a rocket. In the air, as described by Eidam, the "basidium" (Fig. 1 E) becomes separated from the conidium (Fig. 1 D) by the rounding off of the conidium where the point of the "basidium" pushes into it. Very occasionally this separation fails to occur (Fig. 1 F).

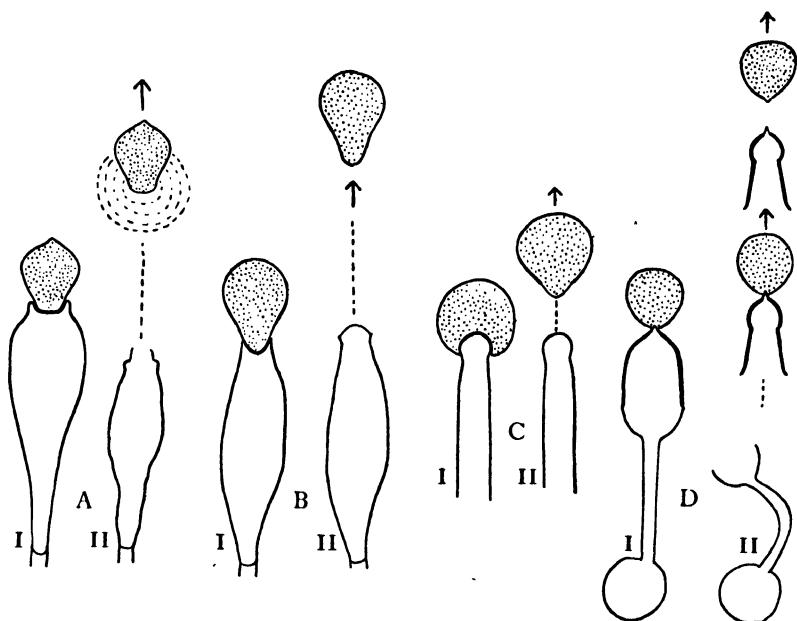


Fig. 2. Diagrams to illustrate types of spore discharge in the *Entomophthorales*. A, *Empusa muscae*; B, *E. Grylli*; C, *Conidiobolus villosus*; D, *Basidiobolus ranarum*. I before, II at discharge. A and B based on figures and description by Thaxter(4), C on those of Martin(3).

It is of interest to compare the method of discharge in *Basidiobolus* (Fig. 2 D) with that found in the other genera of the *Entomophthorales*. In this order of the *Fungi* violent spore discharge is the rule, being exhibited by all genera except *Massospora*. In *Empusa muscae* (Fig. 2 A) the turgid conidiophore explodes (Thaxter(4)) discharging the conidium together with a drop of conidiophore sap, much after the fashion of *Pilobolus*. In *Empusa Grylli* (Thaxter(4)) the columella is pushed back into the conidiophore by the bulging wall of the spore. On discharge this re-entrant columella is suddenly pushed out by the turgidity of the conidiophore and the conidium is thrown off (Fig. 2 B). In *Conidiobolus villosus*(3) the columella pushes into the conidium.

Spore Discharge Mechanism in Basidiobolus ranarum 277

The spore in this case suddenly rounds off and in doing so bounces away from the conidiophore (Fig. 2 C). It is clear that in the *Entomophthorales* violent spore projection has been evolved along more than one line of development.

REFERENCES

- (1) EIDAM, E. *Basidiobolus*, eine neue Gattung der Entomophthoraceen, in Cohn, *Beitr. Biol. Pflanzen*, **4**, 181. 1886.
- (2) LEVISOHN, I. Beitrag zur Entwicklungsgeschichte und Biologie von *Basidiobolus ranarum* Eidam. *Jahrb. Wiss. Bot.* **66**, 513. 1927.
- (3) MARTIN, G. W. Morphology of *Conidiobolus villosus*. *Bot. Gaz.* **80**, 311. 1925.
- (4) THAXTER, R. The Entomophthoreae of the United States. *Mem. Bost. Soc. Nat. Hist.* **4**, 131. 1888.

POLLEN ANALYSIS. AN OUTLINE OF THE PROBLEMS AND POTENTIALITIES OF THE METHOD

PART I. TECHNIQUE AND INTERPRETATION

By H. GODWIN

Botany School, Cambridge

(With 9 figures in the text)

CONTENTS

	PAGE
Introduction	278
Preparation of samples	280
Identification	281
Differential pollen preservation	289
Calculation and presentation of results	289
Pollen downwash through the soil	294
Pollen spectrum as an index to forest composition	295
(a) Pollen production	296
(b) Wind transport	299
(c) Flotation and water transport	301
(d) Time of flowering	302
References	304

INTRODUCTION

THE first aim of pollen analysis is to establish the pollen content of recent geological deposits. This pollen flora has been derived from surrounding vegetation, especially the wind-pollinated plants, during formation of the deposit. The deposits vary greatly in the extent to which they preserve the pollen, but in the waterlogged anaerobic layers of growing peat beds the pollen membranes are extremely well preserved, and after removal of humic material by alkali or acid oxidation can be readily identified. A very large component of the pollen rain incorporated in these deposits has always been the pollen of the forest trees, for these are anemophilous and over large areas are natural climatic dominants. Pollen analysis has been mainly concerned with the tree pollen however, not only on account of its abundance, but because as vegetation dominants the trees are the most direct indices to past climates, and also as dominants of the natural vegetation must have played a great part in the lives of prehistoric people(14).

Not only are the pollen grains evidence of the former presence of different kinds of trees but counts can establish indices to the relative frequency of different tree genera. "By establishment of these frequency figures layer by layer through the pollen-bearing strata it becomes possible to follow former plant-geographic changes from place to place and from one time period to another" (von Post(27)).

In the vertical sequences through the deposits of a district it is possible to establish fundamental points of similarity and by these in turn to establish horizons of equal age.

Such methods of analysis have been applied both to inter-Glacial(19, 33, 21) and post-Glacial deposits, but it is for the latter particularly that the last twenty years have seen intensive investigation in many parts of the world. Since the pioneer work of von Post it has become increasingly clear that in broad outline the same story of forest movement is recorded from the post-Glacial deposits of all Europe. Extension of the wide body of pollen analysis data continues to confirm von Post's threefold division of post-Glacial time on a climatic basis into (a) the period of increasing warmth, (b) the period of maximum warmth, (c) the period of decreasing warmth. This climatic sequence is strongly indicated by the forest movements. In the period of increasing warmth following the last retreat of the ice-sheets forests of pine and birch advanced northwards across Europe. After them followed the warmth-loving trees such as oak, elm and lime, and during the warmth maximum these trees reached their greatest extent and importance. Towards the end of the period of maximal warmth the beech and hornbeam spread extensively, but, together with the components of the mixed oak forests, showed retrogression in area and abundance in the following period of diminishing warmth, in which the Conifers showed a tendency to return to their former dominance. This story is clearly evident in the small pollen diagram (Fig. 1, after Keller(20)), from central Switzerland. This shows also the marked hazel maximum which occurs over a very large part of Europe at the transition to the period of maximal warmth. Naturally there are great differences in the expression of this forest succession between the north and south of Europe, and some, though smaller ones, between east and west. These general drifts are briefly mentioned in a later section of this paper, but it is not the present aim of the author to collect and analyse the very large mass of data acquired all over Europe by pollen-analysis methods. Despite careful attention to theoretical and practical problems of the methods of pollen analysis by numerous workers in the subject and a

recognition of the extreme caution necessary in interpreting the results it yields, there is no simple general statement of the precise scope and limitations of the method. It is this alone which is intended in the following pages; and though not exhaustive of even this more limited subject, it will present a general picture of what the method can and cannot do, and what technique is likely to be of most profit in developing an intensive study of the British post-Glacial deposits.

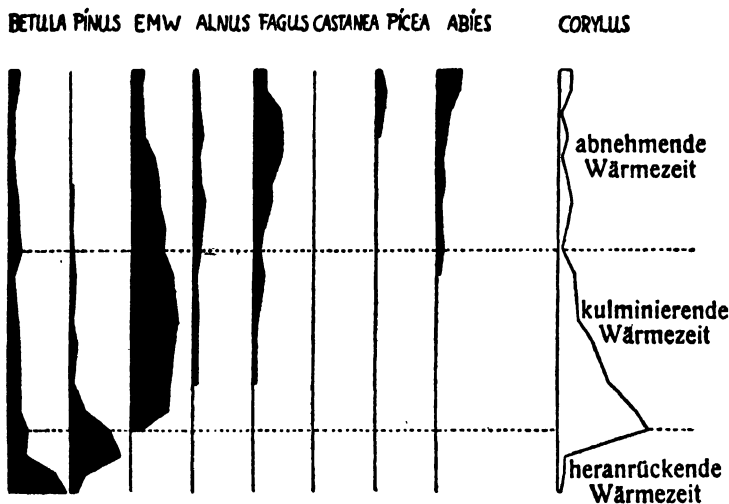


Fig. 1. Diagram from Keller for Switzerland showing division of the post-Glacial into three climatic periods, and the corresponding changes in pollen composition showing altering forest composition. In each vertical column is shown the pollen of a single tree genus expressed as a percentage of the total tree pollen. *Corylus* is also expressed as a percentage of this figure, but does not contribute to it. EMW stands for "Eichenmischwald," the mixed oak forest with *Quercus*, *Ulmus* and *Tilia*.

PREPARATION OF SAMPLES

The usual method of preparing peat samples for analysis is based on treatment with hot alkali. Calcareous marls are treated with hydrochloric acid, and the organic component in siliceous sediments is concentrated by decantation and by treatment with hydrofluoric acid. The alkali treatment of peat is extremely simple: in most cases a small amount of the sample is boiled up on a microscope slide with a few drops of 10 per cent. KOH or NaOH. The macerated material is then examined directly, or it may first be washed and afterwards mounted in glycerine jelly, either unstained or coloured with safranine. Various modifications of this simple technique have been described, mostly with a view to placing the pollen counts on an

absolute basis; a given weight or volume of peat is boiled up with alkali, is repeatedly washed, centrifuged, and finally made up in such manner that a known amount is present on each slide.

More recently G. and H. Erdtman(9) have proposed an alternative maceration method based on mild oxidation followed by acid hydrolysis. The oxidation agent is NaClO_3 in a mixture of acetic and sulphuric acids. It is allowed to act for some hours in the cold and then the residue is washed and dried with acetone and ether. The mass of material so produced is treated with 80 per cent. sulphuric acid which hydrolyses the abundant polysaccharide fraction, leaving a sample extremely rich in pollen grains. This residue is made up in lacto-phenol stained with methylene blue, and a definite volume is placed for counting in a specially made chamber of the type of a blood-corpuscle counter. The authors claim that this method, though yielding results like those given by the alkali method, concentrates the pollen so much more, that analyses can be made of samples far too poor in pollen for alkali preparation.

In the same publication attention is drawn to a common source of error in the final mounting of material. It is shown that pressure on the cover-slip causes over-representation of large pollen grains by the squashing out of smaller grains at the edges.

The same authors show that prolonged alkali treatment causes destruction of the pollen-grain membranes, and suggest that this destruction is differential for different tree genera. Though this is probably so, the evidence suggests on the whole remarkable uniformity in pollen composition up to the final disappearance of all the grains, and it is clear that further evidence on this point is desirable, and that it should be given as results which have been subjected to statistical proof.

IDENTIFICATION

The identification of fossil pollen is based on criteria of size and shape of the grain, number, size, form and arrangement of the germ pores, thickness of the pollen-grain membranes and surface markings or thickenings of various kinds. The genera of British trees which are preserved in peat have pollen grains now so familiar in appearance that it is unnecessary to give any key to their identification, although Fig. 2 has been included so as to show their salient characteristics when seen at different angles and when drawn on a common scale.

There is only one serious possibility of confusion between the different genera. The pollen grains of *Salix* and *Fraxinus* are very

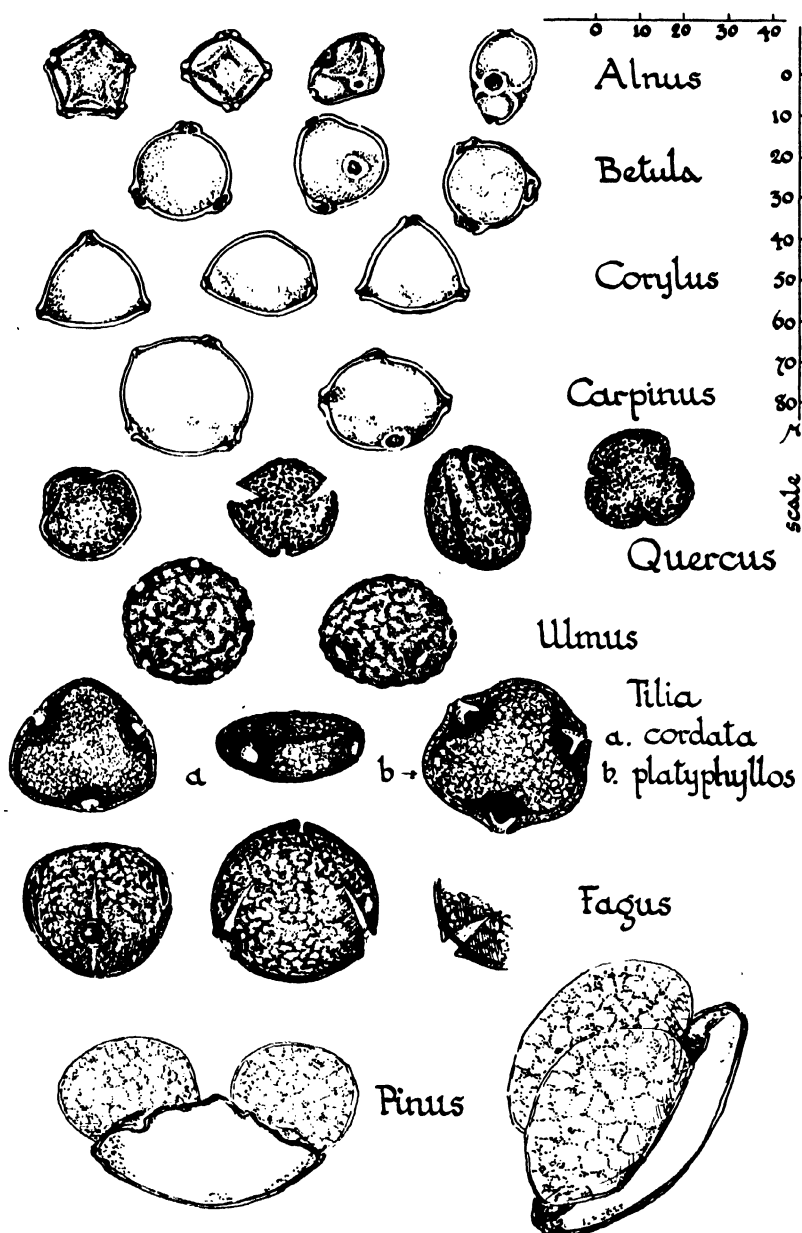


Fig. 2. Diagram to show the salient characteristics of the pollen of the chief genera of trees which are represented in British post-Glacial deposits. The grains are shown as they appear in the subfossil state, they are all drawn to the same scale, and views from different angles are presented so as to give an idea of the solid shape of each grain.

similar to one another in appearance, and though they seem to differ in size the author is not aware of any studies of the size variation in pollen from *Fraxinus excelsior* nor from the numerous species of *Salix* which might be supposed to have left pollen in British post-Glacial deposits. It would seem that until these studies have been made the only means of separating the two genera is to use the minor morphological character suggested by Meinke (25) of the clearer longitudinal folds in *Fraxinus* and to adopt a provisional size limit of about 27μ for length of the grains, regarding the larger ones as *Fraxinus* and the smaller as *Salix*. It is to be noted that the pollen of *Fraxinus* is frequently said not to be preserved; so that the *Salix-Fraxinus* type of fossil grain is for the time being perhaps best spoken of as "salicoid." Because of this uncertainty of identification, coupled with the fruticose habit of many *Salices*, it is not customary to reckon salicoid grains in the total counts of tree pollen, and for this reason also we omit illustration of the grains in Fig. 2. Though the tree pollens are unlikely to be confused with one another yet some of the genera have pollen extremely like that of various shrubs or herbaceous plants to which they may be quite unrelated. Thus the pollen of *Quercus* is not distinguished from that of *Viola*, though there is seldom any doubt that where the pollen is present in quantity and in deposits found some distance from forest sites, the pollen is that of the wind-pollinated tree. Similarly pollen of *Fagus* is indistinguishable from that of *Hippophae*. Since *Hippophae rhamnoides* is not confined to coastal habitats, but appears widely on scree and moraine soils on the continent, there is always the possibility that records of "beech" grains in deposits greatly pre-dating the general beech invasion of a region, may, in fact, be due to *Hippophae*. This is the interpretation given by Thomson (34) to explain the presence of such grains in the subarctic layers of peat deposits in Esthonia, a country which has never been reached by beech forests in the post-Glacial period. The salicoid type of grain is much like that of *Adoxa*, and *Corylus* pollen closely resembles that of the nettle, *Urtica dioica*. Under conditions of prehistoric forest dominance these herbaceous plants probably were less likely to affect the general tree-pollen deposition than under present-day conditions of extremely extensive forest clearing. It is sometimes a matter of difficulty to distinguish between grains of *Corylus* and *Betula*; the usual criteria of distinction lie in the germ-pore characters shown in Fig. 2, the sharply projecting thick ring round the uniform wide pore of *Betula* contrasting with the gradual and slight thickening which surrounds the conical and narrower pore of *Corylus*.

Nevertheless, a small percentage of grains sometimes appear to be intermediate in respect of these characters.

A much more serious possibility of error than this lies in the possibility of confusing pollen of the shrubs, *Corylus* and *Myrica*. It used to be generally held that the pollen of *Myrica* was seldom preserved. Madame Szafer (18), who has been able to work out criteria for distinguishing the two types of grain, says that there is no evidence to suggest that this is so. The chief distinguishing characters are: (1) *Myrica* has a thicker exine; (2) the exine thickens near the pores in *Myrica* whilst it is equally thick in *Corylus*; (3) there is only one thick layer in the exine of *Myrica* but there are two in *Corylus* (besides other thin layers); (4) in *Myrica*, on the inner surface of the exine and extending a short way in from each pore, the thickening is visible in optical section as a bright area on the wall. .

Though Madame Szafer's paper was published in 1928 the problem of recognising separately the pollen of *Corylus* and *Myrica* is unrecognised in subsequent pollen analysis papers; this is partly due, no doubt, to the difficulty of distinguishing the two pollens and partly to the continued belief that *Myrica* pollen is easily destroyed. Fortunately the "coryloid" pollen is not reckoned in total tree pollen, but in view of the importance of the boreal hazel maxima (8) and in view of the widely different edaphic requirements of *Corylus* and *Myrica* and therefore of the inferences to be drawn from abundant pollen of either of them, it is very undesirable that the problem should be left at this stage.

Though the tree *genera* are separable by their pollen, in most cases the *species* are not. Thus *Quercus robur* and *Q. sessiliflora*, though probably differing in size, are otherwise alike, and there is no means of distinguishing the pollen of the separate species of *Betula*, *Salix* or *Ulmus*, nor do the systematic relationships within these genera suggest that such a means is likely to occur. The size variation of the *Betula* species has been worked out by Madame Szafer and others, and Fig. 3 gives some idea of the range involved. This is a diagram by Bertsch in which the thick line shows the size of fossil pollen from the birch phase of the Federsee pollen diagrams. From the form of this curve Bertsch concludes that *Betula nana*, *B. pubescens* and *B. verrucosa* were all present in this area and contributed to the birch pollen maximum. *B. humilis* may also have been present but could hardly have alone accounted for the major peak of the fossil pollen size curve. Since only one species of *Alnus* occurs in Britain the pollen is unlikely to be attributable to others, but it should be remembered

when comparing British pollen analyses with continental results, that in the latter *Alnus* pollen includes not only that of *A. rotundifolia* but also of *A. incana* and *A. viridis*, which are indistinguishable from it.

In the same way, although it has been shown that the pollen of different species of pine are distinguishable from one another (12-15), the total pine pollen curve in most continental diagrams includes the pollen of *Pinus montana* and *P. cembra* as well as *P. sylvestris*. Erdtman suggests(7) that it is partly on account of *P. montana* pollen that the pine maximum in many parts of the continent falls so much earlier than in England (i.e. in the Early Boreal or pre-Boreal). Care is

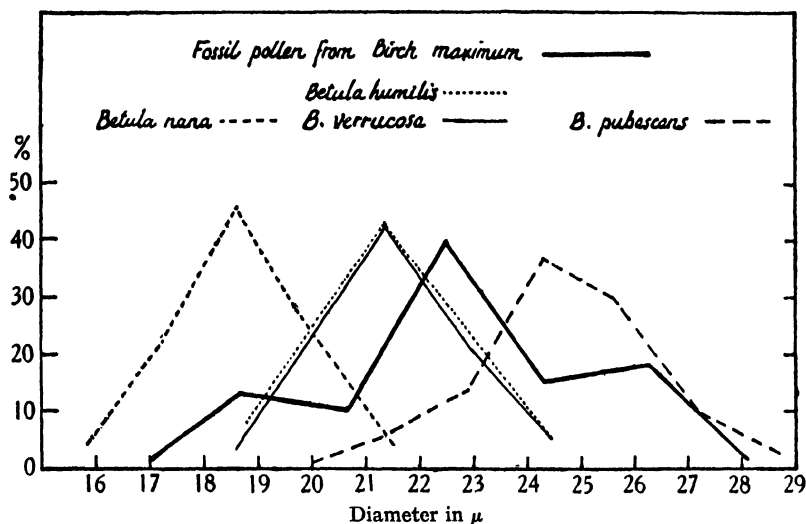


Fig. 3. Size variation curves for recent pollen of species of *Betula* compared with a similar curve for fossil birch pollen from the birch maximum in the Federsee region. After Bertsch(3).

therefore necessary in comparing the British pine pollen curves, due solely to *P. sylvestris*, with those of the continent.

In the case of *Tilia* alone of the British tree genera is it possible to distinguish the species. It has been shown by Trela(35) that *T. cordata* Mill. and *T. platyphyllos* Scop. have pollen which can be readily distinguished by the following criteria. The grains of *T. cordata* are triangular in outline, the germ pores lying in the middle of the sides, but *T. platyphyllos* has hexagonal grains with the germ pores at alternate corners. The grains of the latter alone show the outline projecting as a conspicuous peg through the base of the germ-pore cavity. Finally Trela gives the distribution curves for the diameters of the grains of the two species, both recent and fossil (Fig. 4). Our

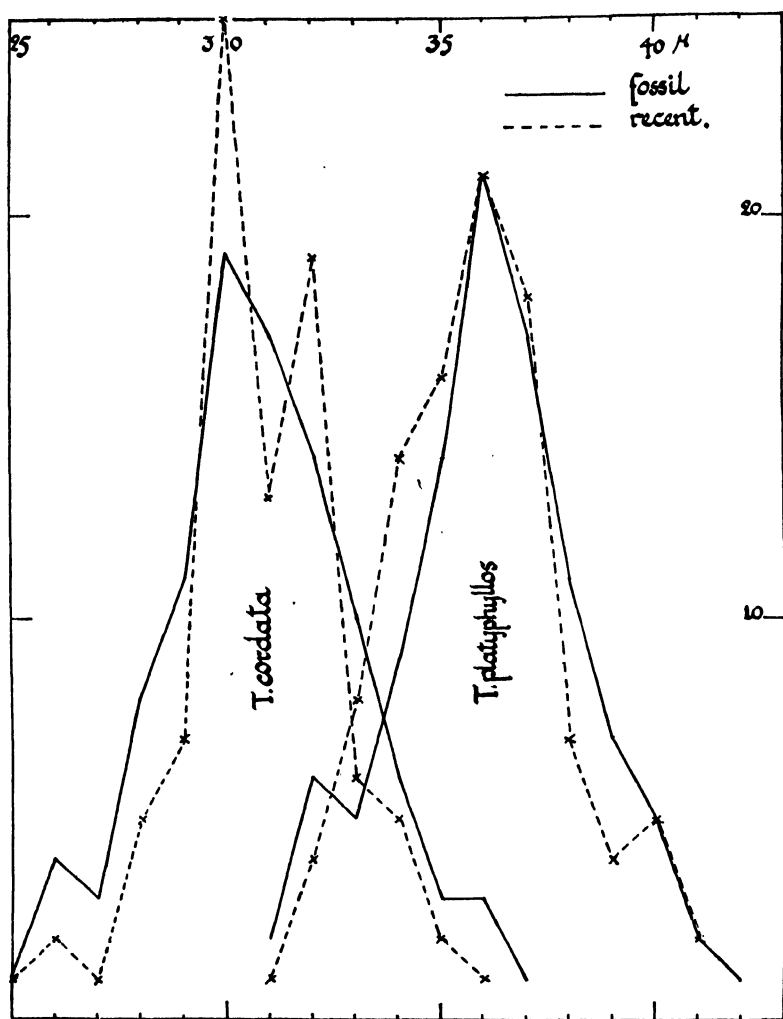


Fig. 4. Size variation curves for recent and fossil pollen of *Tilia cordata* and *T. platyphyllos*, after Trela(35). Based on measurements of samples of 100.

own preparations have shown that *T. europea*, which is usually considered a hybrid between *T. cordata* and *T. platyphyllos*, has pollen grains of intermediate morphological character, and, as will be seen from the graph of Fig. 5, also of intermediate size. The respective modes and ranges for the curves are:

	Range in		Mode in	
	Recent	Fossil	Recent	Fossil
<i>Tilia cordata</i>	25-37	25-37	31	31
<i>T. platyphyllos</i>	31-41	31-42	36	36
<i>T. europea</i>	28-42	—	34.5	—

In the fen and coastal deposits of East Anglia *T. platyphyllos* and *T. europea* are almost entirely absent, but pollen referable to *T. cordata* on morphological grounds is locally quite abundant, and, as will be seen from the curve in Fig. 5, it has a modal size value of 27-30 μ .

It should be noted that the type and media of maceration and mounting have been shown to affect the size of the grains, and such a factor may be partly responsible for the differences in modal size in *T. cordata* pollen from Poland (Trela) and from the fenland peats. The discrepancy is so large, however, that it clearly shows the desirability of wider information as to the factors which may affect the size of pollen grains, not only factors of technique and preparation but the effects of phenotypic and genotypic factors acting through the parent trees.

The identification of spores and pollen grains of plants other than trees almost always accompanies and reinforces the routine estimation of tree pollen. In this task the pollen atlas and key prepared by Meinke⁽²⁵⁾ for German bog plants is of great value, though each investigator will naturally build up for himself a type collection of recent pollen and spores. The non-tree pollen is of particular interest as key to the successional development of a bog. Some large families such as Cyperaceae and Gramineae have grains which cannot be separated into species except by size, and since each family has species which occur over an extremely wide range of habitats, their pollen has no great indicator value. In this respect the tetrads of the Ericaceae are much more valuable, since the family is on the whole restricted to acid soils. Overbeck has recently added also a systematic comparison of the grains of the middle and north European species of Ericales⁽²⁶⁾.

Though valuable as indices to local conditions, the non-tree pollens have for obvious reasons far less general use as climatic or chronological indices than those of the trees.

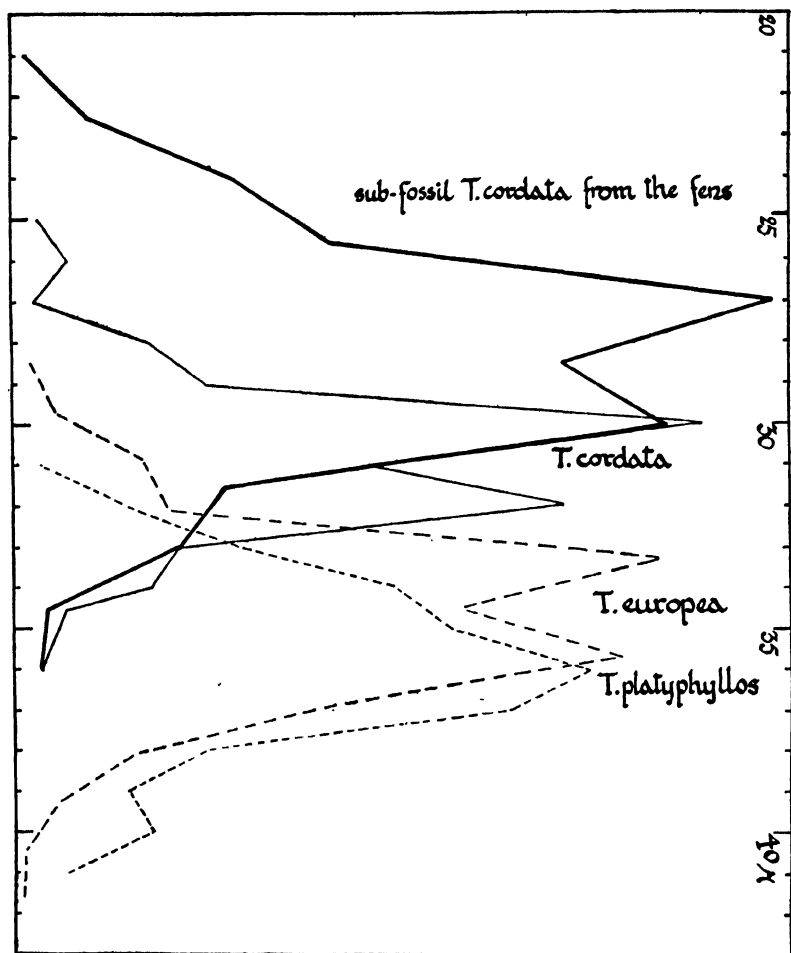


Fig. 5. Size variation curves for sub-fossil pollen of *Tilia cordata* from post-Glacial deposits of East Anglia, from measurements of 431 grains. For comparison size variation curves are given for recent pollen of *T. cordata* and *T. platyphyllos* (after Trela), and of *T. europea*. The latter curve is based on measurements of 302 grains taken from a mixture of the pollen of twelve separate trees.

DIFFERENTIAL POLLEN PRESERVATION

The processes involved in pollen decay are presumed to be both bacterial action and direct oxidation. Pollen is best preserved in waterlogged and unaerated deposits both of mineral and organic origin. In the former class the clays and fine silts often contain well-preserved pollen though identification of the grains is often made difficult by the mineral debris. In coarser material preservation is poor and identification generally impossible. It is in peat that preservation is most complete, though the acid peats are far better in this respect than the alkaline peats of topogenous bogs such as those which characterise the fens and broadland of East Anglia.

Considerable differences exist between different tree pollens in their capacity to resist destruction. Pollen of *Populus*, *Taxus*, *Juniperus* and members of the Rosaceae is easily destroyed, but fortunately that of all the forest dominants is relatively well preserved. Some differences in destructibility may well exist between these, but it is difficult to see how such differences can be evaluated. Over certain genera opinion is conflicting; thus *Fraxinus*, *Myrica* and *Acer* are sometimes quoted as having no pollen preserved in peat, whilst they appear in the analyses of other workers. *Larix* pollen is rather readily destroyed and it appears chiefly in horizons where macro remains such as stubs and cones lead one to think that *Larix* must have had great local density; even in these places the percentage of larch pollen is quite small (Firbas⁽¹⁰⁾). Hesmer⁽¹⁵⁾ has also suggested that the pollen of *Quercus* is more susceptible to decay than that of other tree genera.

In samples which have suffered much decay it seems very probable that even if destruction affects all the grains equally, the analyses will favour those genera with very readily recognisable pollen, such as *Alnus*, at the expense of other genera such as *Quercus*, which in a crumpled or fragmentary condition are much more difficult to recognise.

CALCULATION AND PRESENTATION OF RESULTS

From the commencement of pollen analytic investigation it has been customary to count, in all but the most difficult samples, a minimum number of 150 grains of tree pollen. Pollen of *Corylus-Myrica* type is not reckoned in this total. It has been demonstrated that 150 is the minimum number necessary to reduce sampling error to sufficiently below the magnitudes of changes in pollen composition

it is desired to measure, and some statistical observations apparently support this view(2).

The amounts of pollen of different tree genera are expressed as percentages of the total tree pollen, and the *Corylus-Myrica* pollen is expressed also as a percentage of this total. It is a method of obvious disadvantages, since a real variation in the pollen rain in respect of a single genus must always affect the percentage values for all the others. For this reason it would be much preferable to express the amount of each pollen type on an absolute scale. The difficulties in achieving this are quite insuperable, however. Fresh weight or volume bases are excluded by the extremely high and variable water content of peat samples. Dry weight, ash or carbon content, which might at first glance seem suitable, are also unsuitable since they are all affected by variable plant composition, by varying rate of peat growth and varying rates of subsequent destruction. The fundamental absolute value it is desired to investigate must be the pollen rain (or rather pollen incorporation) for each type *per unit time*, but the variations in rate of formation, in material of formation and in degree of subsequent decay and compacting stand in the way of obtaining any present indices to this absolute scale. It is not even permissible to assume that the same net rate of peat formation is maintained in any single peat bog, since the processes of vegetational succession themselves (to say nothing of external climatic or geological factors) will cause changing rates of peat formation vertically throughout the bog, as do the vegetational zonations horizontally. The presence of tussocks and hollows and varying types of plant cover make it probable that the rate of peat formation will even vary foot by foot across the surface of a bog.

Though absolute pollen frequency is liable to such variation within adjacent samples that it is not practicable to express the amounts of pollen of separate tree genera in absolute terms, yet rough estimates of absolute tree-pollen content may in some cases be usefully made. Such estimates frequently are indicative of changing edaphic conditions through the development of a bog; thus in the development of peat bed E, at St German's, a much lowered pollen frequency was closely associated with the development of a peat bed into a phase of fen oakwood when the relatively dry conditions of the forest floor might be supposed adequate reason for the intense destruction of pollen and low resultant frequencies. Absolute frequency of total tree pollen based on number of tree pollen per square centimetre of slide preparation surface was used by Erdtman in his

investigations of northern Scotland and the Scotch isles(6). It gave the results shown in Fig. 6 and appears to demonstrate the possibility of a general diminution of past tree cover progressively across the area towards the Atlantic.

Though absolute pollen frequencies for the separate trees are excluded it might still be supposed possible to estimate the variation in percentage composition due to sampling within a series of samples of the same age. It is, however, almost impossible to trace lateral bedding in most peat beds with sufficient accuracy to guarantee the detection of a layer of uniform age, and from what has already been said with respect to local variation in peat growth it will be clear that neither a horizontal plane nor a plane parallel to the base of the bog

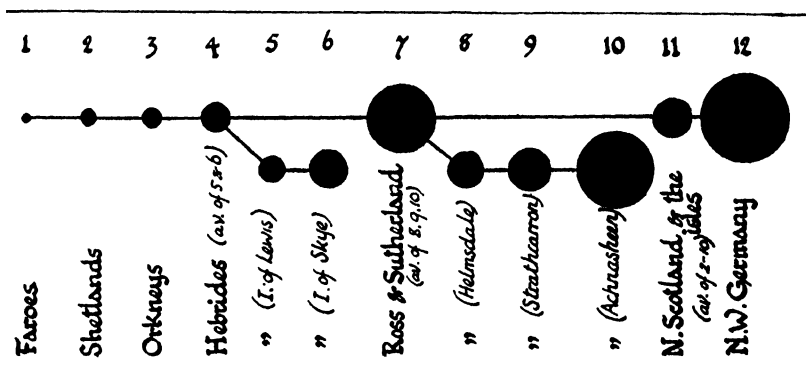


Fig. 6. Diagram after Erdtman(6) showing the diminution in absolute tree-pollen frequency (reckoned as number per unit area of slide surface) north and west from the continent across northern Scotland.

will be quite satisfactory as a surface of contemporaneous peat formation.

An interesting series of observations might be made by exposing a suitable vertical surface in a thick peat bed and by subjecting to analysis samples taken in a close vertical series along each of three or four lines upon the exposed face. By the normal process of analysis similar sequences would become evident in all the vertical series, and these could be used as a rough basis for the establishment on the peat face of lines of equal age. By returning to the protected peat surface with the original sampling points still recorded clearly *in situ* upon it, it should then be possible to secure, along such lines of equal age, samples giving a general knowledge of the variation of tree-pollen content in space across a peat bed. Naturally in such a case it would be necessary to use the greatest care in interpreting the drift of the

pollen curves of the initial vertical series, having in mind as far as possible the whole story of the development of the peat deposit. It is likely that only in a few special cases would peat bogs be susceptible to such experiment, and it seems impossible in the majority of cases to escape from the necessity of expressing the amounts of different tree pollens as percentages of the whole. The chief drawback of this method of expressing pollen analysis results is that there can be no direct indication of the absolute change through a peat bed of the amounts of the pollen of any single tree genus. That is to say, it excludes by implication any direct picture of the changes of absolute density of oak or any other trees growing in the forests of the region throughout the period of formation of the peat. Nevertheless the method will always indicate changes in the *relative* abundance of the pollen of different tree genera. Thus, if oak increases and alder decreases during the formation of a peat deposit, this change will be reflected in the percentage pollen composition of the peat samples throughout the bog, though the absolute values for alder and oak pollen may for various reasons vary very widely. Thus the method of expressing the frequency of each tree pollen as a percentage of the whole can be trusted to reflect changes with time in the percentage composition of the woodlands. The *direction* of such changes will be more clearly shown than the *magnitude*. Nevertheless, when several genera are concerned changes in the relative abundance of the different genera afford extremely clear indication of climatic or edaphic changes, and it is the recognition and evaluation of such changes that constitute the pollen analytic method. That is to say, the method is not one dependent on the comparison of single samples from scattered sites, but rests on the comparison of the drifts evident in percentage composition throughout a number of vertical series of samples. Naturally in such comparisons the level in a series at which any particular tree pollen first appears also ranks as a horizon of great importance, since it may be taken as roughly indicative of the date of immigration of the tree. At the same time, since the pollen of the new species at first forms only a very small part of the total tree pollen, only extremely large or numerous pollen counts can be trusted to record the earliest date of immigration.

In the comparison of the time drifts of the pollen composition curves it is usual to find remarkably close general correspondence in the vertical series from any one locality or region. Thus diagrams *a* and *b* of Fig. 7 represent two separate series of analyses from the face of a peat bog exposed by coastal erosion at Skipsea on the east coast of

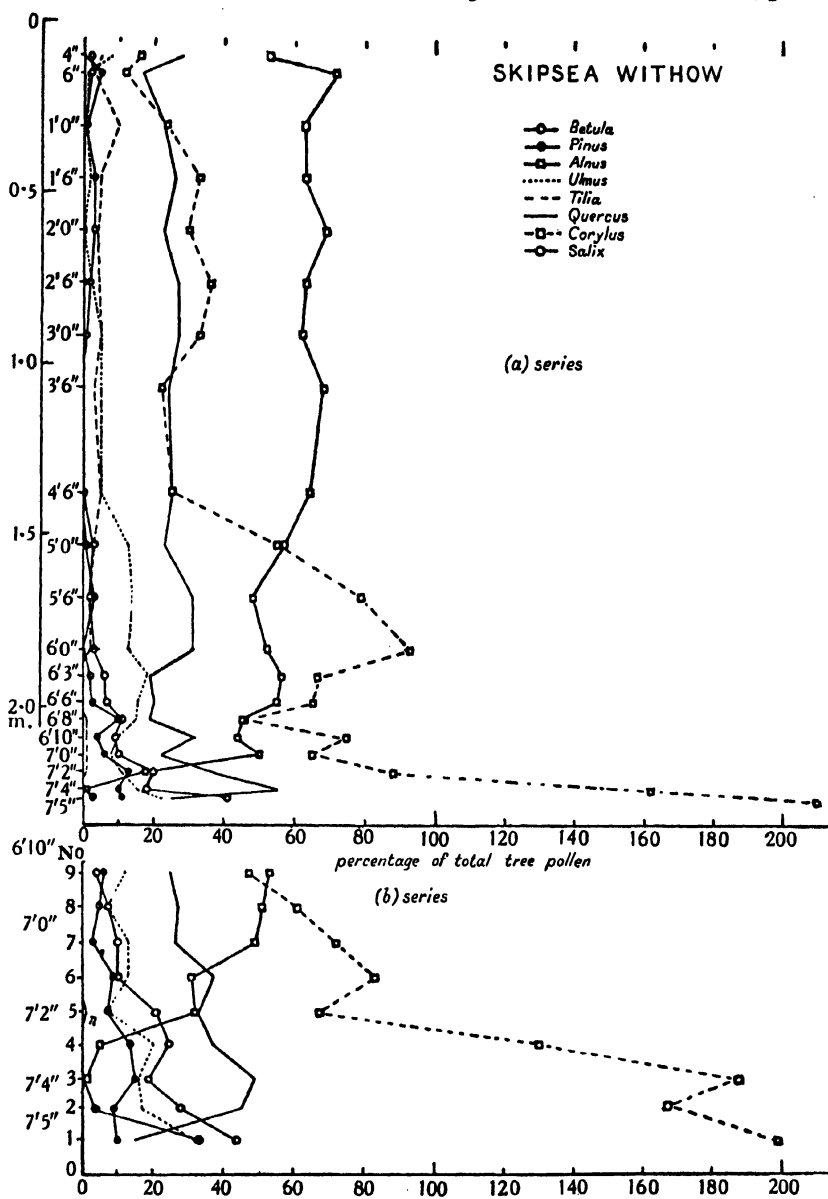


Fig. 7. Pollen analysis of a vertical series of peat samples taken through the bed of an ancient mere now being exposed by erosion on the Yorkshire coast at Skipsea. The upper diagram shows the complete sequence; the lower diagram is of the same site but is derived from a closer series of samples taken through the base of the peat bed some months after the first series. (Reproduced from *Antiquity* by kind permission of the authors.)

Yorkshire(13). Series *a* was collected and analysed with the results shown, and some months later series *b* was collected by a return visit to the original site. The later series was intended to give a more detailed picture of the basal few inches of the peat bed than that given by series *a*, and samples were therefore taken at much closer intervals though within a few inches laterally of series *a*. The correspondence between the two separately analysed series is extremely striking, nor could one mistake from direct inspection the levels corresponding in age in the two series. This is the condition generally found, although, where the comparison is between two series taken some distance apart, the vertical scales through the series may have to be considerably modified in order to get correspondence in the drift of the pollen curves. This is naturally a result of varying rates of peat formation, and though the difficulty it causes can be met by careful consideration of the pollen diagrams, yet it prevents any satisfactory treatment by direct statistical methods.

POLLEN DOWNWASH THROUGH THE SOIL

It has been suggested (Malmström(23)) that downwash of pollen through the soil by descending water, especially along frost cracks, might be a fertile source of error in the pollen analysis method. It is likely to be of least importance in fine subaquatic inorganic deposits and most in open rapidly forming peat. Nevertheless Erdtman's investigations(5) seem to indicate that this source of error is not important even in *Sphagnum* peat areas. Erdtman investigated the pollen content of growing *Sphagnum* tussocks (Bulten) both in their surface layers and also at depths of about 0.5 m., corresponding to the surfaces of the hollows between the tussocks. The surface layers of these hollows (Schlenken), which are apparently not forming peat nor incorporating present-day pollen, were also investigated and were found to have a pollen content exactly comparable with that from the samples 0.5 m. below the surface of the growing tussocks. Such could hardly be the case if downwash of pollen were affecting the peat either of the tussocks or of the hollows.

It has also been pointed out by Erdtman(5) that the great conformity of the drifts in pollen diagrams of deposits formed at very different rates, very strongly opposes the possibility of pollen downwash. If it is to be assumed that pollen downwash has taken place in these deposits, it is necessary also to assume that in each case its effect has been proportional to the rate of formation of the deposit.

There is a good deal of similar evidence against the likelihood of such serious general errors, and Malmström himself later (24) showed, by direct experiment with *Lilium* pollen, that even in peat soils there is no substantial pollen downwash. Nevertheless, the possibility of such error must always be envisaged in interpretations of the presence of scattered pollen grains out of place in the general sequence of results of a regional analysis.

POLLEN SPECTRUM AS INDEX TO FOREST COMPOSITION

It has been already indicated that differential pollen preservation must impair to some degree the accuracy with which the fossil pollen content reflects the composition of former woodland. The error so introduced is clearly indicated in the parallel analyses of wood, pollen and other tree remains shown in the following table.

Remains of woody plants found associated with Bronze Age hut sites at Dullenried (Federsee). After Bertsch(3).

Tree	Wood from hut structure %	Pollen from culture layer %	Various remains (wood, leaves, pollen or fruit)
Populus }		0	+
Salix }	27	0	+
Corylus avellana	23	10	+
Alnus	21	10	+
Fraxinus excelsior	10	0	+
Betula	9	2	+
Fagus sylvatica	3	44	+
Acer pseudoplatanus	3	0	+
Malus (? communis)	0.6	0	0
Carpinus betulus	0.6	0	+
Taxus baccata	0.2	0	+
Quercus	1.5	9	+
Ulmus	0	1	+
Tilia cordata	0		+
T. platyphyllos	0 }	2	+
Pinus	0	7	+
Picea excelsa	0	11	+
Abies	0	4	+
Sorbus aucuparia	0	0	+
Prunus spinosa	0	0	+
Frangula alnus	0	0	+
Sambucus nigra	0	0	+

It is remarkable how far the wood percentages, calculated on several hundred samples, differ from the pollen percentages. Thus hazel, willow and aspen constitute over half the wood total. This is probably due in part to selective gathering for hut construction, a suggestion borne out by the wood analyses of the *piles* of lake dwellings on other sites where quite other trees, such as oak, ash and pine

predominate. From our present viewpoint it is, however, particularly interesting to note that the pollen analyses give no clue to the presence of *Populus*, *Salix*, *Fraxinus*, *Malus* and *Taxus*, which together constitute 41 per cent. of the wood samples.

Results of similar significance are given from several other sites by Bertsch, who also compares charcoal analyses from prehistoric hearths with the contemporaneous pollen analyses. In the latter case it might be expected that there would be less selective gathering of wood, and it does appear that closer correspondence exists here between the indications of the fossil wood and the fossil pollen. It is perhaps of interest to state that the Dullenried sites of the table given above occur at the beech maximum in the generalised pollen diagram of the Federsee region reproduced in Fig. 16 in Part II of this paper.

(a) *Pollen production*

The effects of extreme susceptibility to decay extend, however, to a few relatively unimportant forest trees, and it is reasonable to expect, apart from this, that pollen analysis will afford an immediate key to former forest conditions. Nevertheless, many factors can be envisaged as likely to complicate this simple relationship, one of the most important being the different magnitudes of pollen production in various tree genera. Realisation of this has led numerous workers (Erdtman(5), Rudolph and Firbas(29, 30), Hesmer(15) and Aario(1)) to make direct comparisons between the pollen content of the surface layers of living bogs and the tree composition of adjoining forest areas. Hesmer has summarised their conclusions (which do not agree in detail) in the following series of diminishing pollen production:

Pinus > *Corylus* > *Alnus* > *Betula* > *Carpinus* > *Abies* >
Picea > *Fagus* > *Quercus* > *Tilia*.

The dotted line represents approximately the point of best representation of forest composition by the pollen spectra. It is usually agreed that *Pinus* is always greatly over-represented¹ by its pollen and oak, and lime much under-represented. Whilst these extreme types are readily agreed to, the position of the intermediates on the scale is less well established. This is no doubt owing to the fact that the pollen amounts have to be expressed as percentages in which each component affects the others. Thus pollen spectra from a pine-beech neighbourhood will certainly show beech more under-represented than those from a fir-beech region, and beech would even be *over-repre-*

¹ But see p. 299.

sented in spectra from a beech-oak district. The only effective means of reaching finality in such a series would seem to be in testing it by contrasting each tree in the scale directly with that above and below it, and in ensuring the absence of other genera in appreciable amounts. Suitable regions for testing such pollen production, with the two given genera more or less equally abundant, would certainly often be difficult to find, and the results would be confused by the facts of variable pollen production at different altitudes, in different climates and on different soils, by the different sizes to which trees grow under these varied conditions and the variation in the age at which they commence to produce pollen. Such considerations make clear the very general character of the pollen-productivity series set out above.

A particularly interesting illustration of the results of comparing pollen deposition with contemporary forest composition is seen in the case published by Rudolph and Firbas in 1926 and illustrated by Fig. 8 given below. Superficial peat samples were collected from different altitudes in the Riesengebirge, so that seven samples were obtained between 740 and 950 m. in the forest belt with dominant spruce (*Picea*), abundant fir (*Abies*) and beech (*Fagus*), three samples between 1040 and 1220 m. in the pure spruce belt, six samples at about 1240 m. in the margin between the spruce and mountain pine (*Pinus montana*), three samples from mountain tops in the dwarf pine belt (*P. montana*), and various samples above the upper limits of the dwarf pines.

In samples from the lowest forest belt the spruce pollen is dominant. *Fagus*, though abundant as a tree, gives only 12 per cent. of the pollen, whereas the pine, quite absent from the woods, is represented by distant wind carriage to the extent of 20 per cent. The fir is even less well represented than the beech. Throughout the spruce belt spruce pollen is dominant, but at the margin of contact with the pine belt the pine pollen is by far the most abundant and remains so through the dwarf pine belt. On the treeless mountain tops, however, there is a striking increase in the pollen from the lower mountain zones. This is due to the weakening of local forest influences which now allow the effects of long distance wind transport to appear. Pollen such as that of oak, lime and elm must in these cases have travelled at least 10 km.

It would appear from these results that conclusions from the pollen spectra as to forest composition can only be of a very general character, but it should at the same time be observed how the

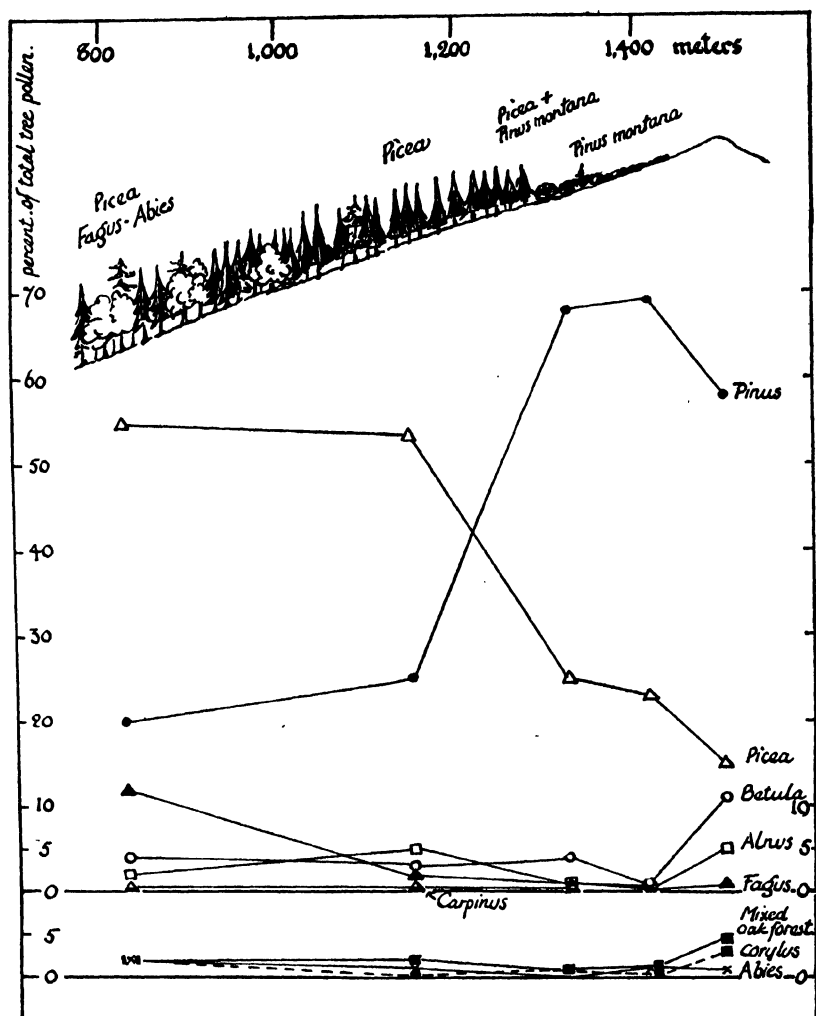


Fig. 8. Diagram to show the degree in which pollen samples from the surface of growing bogs reflect the composition of the surrounding woodland. The upper diagram shows the forest zonation on the Riesengebirge. Below are given the pollen analyses—each of the five results shown is the average of a number of samples taken at a given altitude. Though the beech, spruce and pine curves show their maxima in the proper sequence and roughly at the proper heights, yet the composition of the pollen samples differs very considerably from the forest composition (after Rudolph and Firbas).

maxima of the beech, spruce and pine in the pollen curves do reflect the actual vertical sequence of forest belts.

(b) *Wind transport*

The illustration here quoted from Rudolph and Firbas draws attention to another source of error in deducing forest composition from pollen spectra, namely the variable transportability of pollen of the different tree genera.

Though exact data of the carriage of tree pollen are difficult to obtain there is considerable evidence that they can be transported for very great distances. The data under this head were collected by Hesselmann(16), who quotes the following instances:

Surfaces exposed on light ships in the Gulf of Bothnia at 30 and 50 km. from the coast trapped pollen of spruce, pine and birch. Water samples taken from 5 m. deep in the North Sea and almost 300 km. from the nearest land contained forty pollen grains of conifers per litre.

It has also been shown that plates exposed in aeroplanes at considerable altitudes will trap pollen. The local incidence of rust attack on crops has been shown by Canadian workers to be due to wind-blown spore infection from the south, and rust spores (some still viable) have been found as high as 17,000 ft.(31) In these investigations pollen grains were found abundantly at all heights up to about 10,000 ft., and Dillon-Weston reports similarly upon investigations in England(4).

Probably the most striking instance of long-distance wind carriage is, however, the case quoted by Malmström(24), who exposed plates to trap pollen at Degerö Stormyr and found spruce pollen caught there some weeks before the male cones of the spruce in the neighbourhood first opened. Malmström suggested that this pollen must have been transported from southern Sweden, a distance of 700-1000 km.

It has been very generally held that the winged conifer pollen travels further than that of the Angiosperm trees. Firbas(11) in a recent paper, however, produces evidence which discounts the assumption that the winged conifer pollen is particularly liable to over-representation by long-distance wind transport. He quotes direct measurements of the rate of fall of different tree pollens in still air, from which it appears that though the air sacs do have a large effect, it is offset by the large size of the conifer grains, so that the conifers show somewhat higher rates of sinking than some other species such

as alder. The known cases of very high values of conifer pollen in unwooded regions are attributed to other causes such as the great local preponderance of conifer woods in the region. Where there is no such disproportion in the source of wind-blown pollen Firbas fails to find evidence for increasing percentages of conifer pollen through differential sorting by winds blowing from wooded into woodless regions.

Though these arguments dispose of the possibility of using high conifer pollen percentages as evidence of former low density of tree cover, Firbas suggests that the ratio of non-tree pollen to tree pollen can be utilised as a very efficient guide to this condition, since only in very poorly wooded or unwooded regions does this ratio exceed one. The extremely local distribution of non-tree pollen, however, makes it undesirable to argue from single samples as to the tree poverty of a large area. It is quite clear that if there is differential pollen transport the pollen will be sorted out by winds blowing from the forest margin, and the distance of a deposit from the surrounding woods will be a factor influencing the composition of the pollen rain. Hesmer has proposed the following classification for the various possible components of the pollen rain on a basis of transport distance.

Bog tree component	...	From trees growing on the bog ¹
Marginal ,,	...	From trees on mineral soils round the bog
Regional ,,	...	From the region up to 500 m. from the bog but excluding the margin
Distant ,,	...	From 500 m. to 10 km.
Long distance component	...	Over 10 km.

It is evidently of great importance to determine which components are most strongly represented in any particular analysis; evidence of this may come not only from the pollen data themselves but also from macroscopic fossils, by the stratification of the moor and the knowledge of its development and topography. In most *hochmoor* peat deposits Hesmer considers the Regional component to be far the most strongly represented.

In this fact lies at once the strength and weakness of the pollen analysis method. It does give as a rule a *general* picture of the forest cover over a wide neighbourhood; not merely a parochial picture of the conditions, perhaps highly unusual, in some particular small area on or near the bog. That is to say, the conclusions from a pollen analysis series can be trusted as a rule to convey the general drift of forest

¹ "Bog" is used as a conventional translation of the German "Moor."

history over the whole countryside. The tree pollen, moreover, is produced so abundantly that if it is not destroyed traces at least may be expected of all the genera present.

On the other hand, this smoothing out of detail has its disadvantages, and where long-distance transport is possible over hundreds of miles the presence of scattered grains of any given species cannot be accepted as proof that the species is, or was, growing only in the neighbourhood; for this much less readily movable remains, trunks, branches, fruits and leaves must be identified, and the chances of finding such are often very slight, since many species are seldom to be found living sufficiently close to growing bogs for incorporation and preservation to have taken place.

It will, of course, be recognised that affecting problems of pollen dispersal by wind are such factors as the prevalent wind direction during the period of pollen liberation, and the effects upon wind currents of special topographic features. It is not necessary, however, to say more than that there is evidence for such factors having operated in certain special cases. The suggestion has also been made that the height of the pollen-producing "flowers" upon the tree must influence their dispersal and also the extent to which the grains cling together in groups of two or more. In view of the extreme heights at which pollen has been caught it seems probable that the first of these two factors may have a very slight influence indeed. Distribution of pollen in groups is particularly of importance in that single large groups may occur in a single peat sample, thus causing wide divergence from the normal composition; Erdtman has stressed this possibility, especially for *Alnus* and *Betula*, portions of the catkins of which are frequently found in peat.

(c) Flotation and water transport

It is probable that rivers bring into lake- and flood-plain deposits considerable amounts of pollen, especially from trees such as *alder*, which grow abundantly on the river banks. Auer (see Hesmer (15)) has also shown that pine pollen can be transported in this way. The conifer pollen appears to differ considerably from Angiosperm pollen in its powers of flotation, and where present in considerable amounts forms the conspicuous "Seeb Blüten" of continental lakes. Winds and currents carrying the surface water to the shore would tend to raise the conifer pollen percentage in the shore deposits compared with those forming in the centre of the lake. Certain observations by Malmström and Lundquist (22) appear to support this view, but as is

pointed out by Wasmund(36) and Hesmer the complexity of the factors involved in fossilisation forbids any prediction of over- or under-representation for conifer pollen in particular parts of the lake deposits.

(d) *Time of flowering*

The pronounced maximum for hazel pollen shown at the base of the Skipsea diagrams (Fig. 7) is an extremely frequent phenomenon in British and continental peat deposits and has led to the suggestion that at certain times in the post-Glacial period, especially in the Boreal period, the hazel formed real forests dominating extensive areas of Europe. This extremely interesting possibility has been reviewed by Erdtman(8), who published the accompanying map (Fig. 9) to illustrate the periods of post-Glacial time at which hazel pollen maxima have been found in the various parts of Europe where pollen analyses have been made.

It will be seen that the boreal hazel maxima especially characterise central and western Europe, the British Isles have hazel maxima at all parts of the post-Glacial time scale, there are no boreal maxima in the zone of the Vistula-Dniestr, which stretches from the Baltic to the Black Sea, and east of this the pollen maxima are post-Boreal. In the Baltic the maxima occur mostly in the Atlantic period.

Erdtman draws attention to the extremely early date of flowering of the hazel, and suggests that this becomes of great importance with regard to pollen incorporation in peat-forming bogs. Bogs which are still frozen will, he suggests, be incapable of retaining the pollen blown on to them, it will not be washed down to the wet anaerobic layers of the bog and will decay, leaving no trace in the pollen content of the bog of this rain of hazel pollen. Such, he considers, may have been the explanation of the absence of the hazel maxima from the Vistula-Dniestr zone, where, especially in the boreal period, continental climate may be supposed to have prevailed with accompanying late springs and long-frozen bogs. Similarly the pronounced hazel maxima at all parts of the post-Glacial period (after the subarctic) in the British Isles may be linked with the early springs which accompany a maritime climate. The climatic optimum Erdtman supposes also to have been characterised, in all but eastern Europe, by early springs, and since this optimum occurred in the Boreal in central Europe and in the Atlantic in southern Sweden, this, again, coincides

with the suggestion that the hazel maximum may be a reflection of early springs and active bog growth.

It would clearly follow from the arguments thus advanced that on the one hand a hazel pollen maximum is not necessarily a criterion of a corresponding fluctuation in the importance of hazel *forest*, and, on the other, that hazel may be present abundantly without leaving indications of this in the hazel pollen record of the local bogs.

It is evident that the pollen analysis data must not, in this case at least, be interpreted without careful consideration of other lines of evidence, particularly those relating to climate. The hazel pollen maximum of central Europe is, however, valuable as a chronological index whether it does or does not accurately reflect a phase of forest history, because in either case it seems the response to a widespread climatic change.

REFERENCES

- (1) AARIO, L. Pflanzentopographische und paläographische Mooruntersuchung in N.-Satakunta. *Comm. Inst. Forestalis Fenniae*, **17**. 1932.
- (2) BARKELEY, F. The statistical theory of pollen analysis. *Ecology*, **16**. 1934.
- (3) BERTSCH, K. Paläobotanische Monographie des Federseerieds. *Bibliotheca Botanica*, **103**. 1931.
- (4) DILLON-WESTON, W. A. R. Observations on the bacterial and fungal flora of the upper air. *Trans. Brit. Mycol. Soc.* **14**. 1929.
- (5) ERDTMAN, G. Pollenanalytische Untersuchungen von Torfmooren und marinen Sedimenten in Südwest-Schweden. *Arkiv för Botanik*, **17**. 1922.
- (6) ——— Studies in the micropalaeontology of post-Glacial deposits in northern Scotland and the Scotch Isles, with especial reference to the history of the woodlands. *Journ. Linn. Soc.* **46**. 1924.
- (7) ——— Some aspects of the post-Glacial history of British forests. *Journ. Ecol.* **17**. 1929.
- (8) ——— The Boreal hazel forests and the theory of pollen statistics. *Journ. Ecol.* **19**. 1931.
- (9) ERDTMAN, G. and H. The improvement of pollen-analysis technique. *Svensk Bot. Tidskrift*, **27**. 1933.
- (10) FIRBAS, F. Die Vegetationsentwicklung des Interglazials von Rinnensdorf. *Abh. Natur. Ver. Bremen*. 1932.
- (11) ——— Über die Bestimmung der Walddichte und der Vegetation waldloser Gebiete mit Hilfe der Pollenanalyse. *Planta*, **22**. 1934.
- (12) GERASIMOW, D. A. On the characteristics of the pollen of *Larix* and *Pinus cembra* in peat. *Geologiska Fören. Förhandl.* **52**. 1930.
- (13) GODWIN, H. and M. E. British Maglemose Harpoon Sites. *Antiquity*. 1933.
- (14) GORDON CHILDE, V. The Forest cultures of Northern Europe: a Study in Evolution and Diffusion. *Journ. Roy. Anthropological Inst.* **61**. 1931.
- (15) HESMER, H. Die natürliche Bestockung und die Waldentwicklung auf verschiedenartigen märkischen Standorten. *Zeit. für Forst- und Jagdwesen*, pp. 10–12. 1933.
- (16) HESSELMANN, H. Beobachtungen über die Verbreitungsfähigkeit des Waldbaumpollens. *Meddelanden från Statens Skogsförsöksanstalt*. 1919.

- (17) HÖRMANN, H. Die pollenanalytische Unterscheidung von *Pinus montana*, *silvestris* und *P. cembra*. *Österr. Bot. Zeitschr.* **78**. 1929.
- (18) JENTYS-SZAFER, J. La structure des membranes du pollen de *Corylus*, de *Myrica* et des espèces européennes de *Betula*, et leur détermination à l'état-fossile. *Bull. Int. Acad. Polonaise Sci. et Let. Sér. B.* 1928.
- (19) JESSEN, K. and MILTHERS, V. Stratigraphical and palaeontological studies of interglacial fresh-water deposits in Jutland and north-west Germany. *Danmarks Geologiske Undersøgelse*, **2**, 48. 1928.
- (20) KELLER, P. Die Wald- und Klimageschichte des Fürstenlandes. *Arbeiten aus der Prähistorischen Abteilung des Historischen Museums, St Gallen*, **1**. 1934.
- (21) LOUMAN, G. G. On the occurrence of interglacial (Risz-Würm) peat in Holland. *Proc. koninklijke Akademie van Wetenschappen te Amsterdam*, **37**. 1934.
- (22) LUNDQUIST, G. Methoden zur Untersuchung der Entwicklungsgeschichte der Seen in Aberhalden. *Handbuch d. biol. Arbeitsmethoden*, **9**, 2. 1925.
- (23) MALMSTRÖM, C. Om den pollenanalytiska metoden för åldersbestämning av torvmosslager och dess biologiska förädlingsättningar (föredrag). *Geol. Fören. Förhandl.* **42**. 1920.
- (24) — Degerö Stormyr. Eine botan., hydrolog. u. entwicklungsgesch. Untersuchung eines Nordschwedischen Moorkomplexes. *Mitt. a. d. Forst. Versuchsanstalt Schwedens*, **20**. 1923.
- (25) MEINKE, H. Atlas und Bestimmungsschüssel zur Pollenanalytik. *Botanisches Archiv*, **19**. 1927.
- (26) OVERBECK, F. Zur Kenntnis der Pollen mittel- und nord-europäischer Ericales. *Beih. z. Bot. Centralbl.* **51**. 1934.
- (27) VON POST, L. Pollenanalyse. *Reallexikon der Vorgeschichte*. Berlin.
- (28) RUDOLPH, K. Die bisherigen Ergebnisse der botanischen Mooruntersuchungen in Böhmen. *Beih. z. Bot. Centralbl.* **45**. 1929.
- (29) RUDOLPH, K. und FIRBAS, F. Paläofloristische und stratigraphische Untersuchungen böhmischer Moore. Die Hochmoore des Erzgebirges. *Beih. z. Bot. Centralbl.* **41**. 1925.
- (30) — — — Pollenanalytische Untersuchung subalpiner Moore des Riesengebirges. *Ber. d. Deutsch. Bot. Ges.* **44**. 1926.
- (31) STAKMAN, E. C., HENRY, A. W., CURRAN, G. C. and CHRISTOPHER, W. N. Spores in the upper air. *Journ. Agric. Res.* **24**. 1923.
- (32) STARK, P. Ueber die Zugehörigkeit des Kiefernpollens in verschiedenen Horizonten der Bodenseemoore. *Ber. d. Deutsch. Bot. Ges.* **45**. 1927.
- (33) SZAFER, W. K. The oldest interglacial in Poland. *Bull. Int. Acad. Polonaise Sci. et Let. Sér. B, Nat. Sci. (1)*. 1931.
- (34) THOMSON, P. W. Die regionale Entwicklungs-Geschichte der Wälder Estlands. *Acta et Comm. Univ. Tartuensis*, **A, 17**, 2. 1929.
- (35) TRELA, J. Zur Morphologie der Pollenkörner der einheimischen Tilia-Arten. *Bull. Int. Acad. Polonaise Sci. et Let. Sér. B.* 1928.
- (36) WASMUND, E. Pollenregen auf Ostholst-Seen und seine Bedeutung für die Pollenanalyse. *Centralbl. f. Min. etc.* 1931.

COMMENTS ON RECENT STATEMENTS REGARDING THE NATURE AND ORIGIN OF THE ANGIOSPERMIC STIGMA

By J. McLEAN THOMPSON

"A man has a scientific judgment only on such objects as he is acquainted with through his own researches; how many may there be who have ever once attempted to form an independent opinion of the nature of the organs of propagation of plants by the investigation of their development on only one single plant?"

J. M. SCHLEIDEN, *Principles of Scientific Botany*, p. 374 (1849).

IN a recent article entitled "The Nature and Origin of the Stigma"¹ Dr H. H. Thomas invites criticism of his view on the history of flowering, and challenges those who hold opinions other than his own to reconsider and state the foundations of their beliefs.

Response to this invitation might, meantime, be withheld but for statements by Dr Thomas which call for correction.

It is first necessary to examine the approach of Dr Thomas to angiospermic flowering as revealed in his earlier writings, and in particular to comment upon his statement to the Linnean Society of London on "The Old Morphology and the New." Following a communication upon "The Early Evolution of the Angiosperms" in which it is affirmed that "there seems to be little prospect of solving the problems of the origin of the Angiosperms from the comparative study of modern forms," and that "we must search through the remains of fossil floras for clues to possible solutions," Dr Thomas turns to the Jurassic flora.

He adduces the Caytoniales as having "evolved angiospermy in Lower Jurassic times, apparently by the closing of a cupule-like envelope around the ovules and the formation of a stigma." Some similarity to both Pteridosperms and Angiosperms is indicated, and on this alone comparisons are offered between the cupule-like envelopes of the seeds of *Caytonia* and the modern carpels of Ranunculaceous and Rosaceous species. As to the validity of such comparisons there is no evidence; nor is the implication that the problems of angiospermy are insoluble on the testimony of Angiosperms themselves justified.

¹ *The New Phytologist*, 33, No. 3, June, 1934. Note. The reader is referred to the bibliography given in Dr Thomas's paper.

A second step in approach to the problems of angiospermic flowering is revealed in "The Old Morphology and the New." And here, following pronouncements on subjective and objective reasoning, and the statement that "most scientists aim at an objective approach to their objects of study," it is shown that Dr Thomas has become aware of new conceptions of flowering already expressed by others from their direct study of living Angiosperms. He constitutes himself the voice for such conceptions and proceeds as follows. "In groups like the Ferns, Lycopods, and certain Gymnosperms... both ontogenetic study and vascular anatomy have been shown to have very considerable significance, and there is every reason to suppose that the same is the case in the Angiosperms."

If, with this supposition before him, Dr Thomas had joined with others in the study of angiospermic ontogeny, he would not have relapsed, as he did with startling rapidity, into what he had deplored as subjective reasoning in seeking a more intelligent view of floral evolution. For his statement proceeds, "I have already suggested the derivation of the carpels of the *Caltha* type from the Pteridosperms *viâ* a structure of the *Caytonia* type." The cupule-like envelopes of the seeds of *Caytonia* become, without further evidence, cupules, reduction of the *Antholithus Arberi* type of the Caytoniales to a single stalked anther is conceived, and the type of stamen possessed by *Ranunculus* is reached.

A more subjective approach to modern angiospermy may not readily be imagined, and while "neither delighting in wild speculations nor enjoying an outburst of iconoclasm," Dr Thomas leaves his audience to form their own conclusions as to the bearing of the Caytoniales on the numerous problems of modern angiospermy.

In commenting at this stage on the approach to modern angiospermy herein revealed, the present writer, from personal contact with floral development and adult structure, remarked that "no greater dis-service could be done at the present stage to our appreciation of the problems of the living Angiosperms than to accept readily the reading of their history in terms of ancient floras. The true service of palaeontology to the history of the angiospermic flower will begin with the provision of undoubted Angiosperms, with their record of reproduction, from the rocks themselves."

A third step has now been taken by Dr Thomas in "The Nature and Origin of the Stigma." A subjective outlook is again deplored, direct observations on angiospermic flowering are swept aside, postulates (which, it is presumed, are bases of argument laid down as well

known or too plain to require proof) are provided, and the view that the angiospermic carpel has arisen from a *pair* of concrescent cupules is now advanced. The margins of the inner surfaces of the cupules provide the modern stigma, placentae are fertile branch tips originally surrounded by a cupule, and it is stated that this view agrees with what we know of the ontogeny of carpels.

On these and other points comment may now be offered.

And firstly, following a statement that some, if not all, of the old canons of floral morphology may eventually prove to be well founded, but if so, they must be based on arguments differing radically from those which have hitherto been advanced; that, "at the outset we must do our utmost to avoid a subjective outlook," and that the relationships of the parts of different flowers must be viewed from the standpoint of their possible evolutionary origin, and not merely as parts of a mental process, the theme runs as follows: "It may be argued that there is little difference in practice between a subjective and an objective approach, but this is not true. The subjective treatment of morphology regards form as an intuitively apprehended concept, and consequently considerations of physiology and phylogeny have no practical significance. Crudely stated, the *Caltha* carpel is regarded as a folded leaf, because it looks like one. The objective attitude takes the material of which the plant is built, and considers it in relation to the processes going on within it and to its environment."

It may here be remarked, with equal crudity of expression, that the envelope of the seed of *Caytonia* may be, and is regarded by Dr Thomas as a cupule, because to him it may look like one. Consideration of processes within and without the cupule-like envelope is for ever debarred.

So much for the objective approach as exploited by Dr Thomas in matters of floral morphology, and for argument "differing radically" from that which he has claimed to expose.

Turning now to his statement on the foundations of a system of morphology, he affirms that "all modern systems of taxonomy rest on one fundamental proposition, viz. that the different parts of the flower represent modified foliage leaves." Refutation of this statement is scarcely necessary, but is here made in view of what is declared to be its corollary, namely "that all the floral parts are referable to an original and continuous spiral sequence even though they show no trace of a regular sequence in their ontogeny." As to how a spiral sequence of floral parts must constitute a corollary from any belief

as to the origin of floral organs is not self-evident. It may further be remarked, for it is in accordance with the truth, that spiral succession of parts is widespread in modern angiospermic flowering; it may be observed by anyone engaged in ontogenetic study and is no assumption such as Dr Thomas has seen fit to suggest.

But his statement proceeds as follows. "The consequences of such an assumption are illustrated in a recent contribution to the discussion of carpel morphology. In this paper Prof. McLean Thompson assumes a spiral succession of the structures we are accustomed to call the placentae of the carpels. . . . Mere numbering of primordia in diagrams affords a poor foundation for a far-reaching theory."

Correction of this statement is necessary, for at no point in his "Theory of Scitaminean Flowering" has the present writer assumed or even suggested a spiral succession "of the structures we are accustomed to call the placentae of the carpels." Nor has he advanced a mere numbering of primordia in diagrams as a foundation for any theory. He had followed the entire ontogeny of each Scitaminean species described, has observed the spiral succession in each species from bract to final styler component, has traced with care the rise to prominence of the placentae beneath the styler components, and has made it abundantly clear that the placentae *do not* arise in spiral succession. He has numbered in diagrams the primordia of floral parts, species by species, in accordance with the observed succession alone.

Further obvious deductions advanced by Dr Thomas from his statement on modern systems of taxonomy are that the follicle is the most primitive carpel type, and that no carpel or group of carpels can be regarded as primitively terminating the apex of the flower. These deductions are dispatched as based on unsubstantial grounds.

It will be shown later that neither the interpretation of the follicle nor the terminal position of a carpel or group of carpels depends on a belief in the foliar origin of floral parts.

Having satisfied himself of the insecurity of an alleged fundamental proposition and its alleged corollaries, Dr Thomas states that "it thus becomes necessary either to produce fresh fundamental evidence or to set aside these theories, with all that was derived from them, as assumptions which cannot be proved, and to endeavour to build up a new system."

The re-entry of the Caytoniales into the field of discussion is clearly indicated, and is heralded by postulates for any system of morphology. In brief, the primary postulates offered are as follows: (1) that vascular plants have evolved from simple ancestors, (2) that

the earliest differentiation of ancestral forms was that of reproductive from somatic cells, and (3) that structural or physiological mutations advantageous to the race tend to be selected.

The immediate bearing of these profundities on the matter in hand is obscure. They, however, lead Dr Thomas to suspect the present writer of belittling hereditary characters because he has studied time-factors in growth, the form, state and stature of floral receptacles, and the number, form, stature and distribution of floral organs and what occurs within them, and accepts each form of flower as an individual expression of the problem of flowering. It may rightly be remarked that if, in recording meristic variations, the present writer has assailed inheritance, something should be done about it, and that neither the existence of seed-bearing plants with cupule-like appendages in Jurassic times, at present known, nor arguments based thereon may serve to mask the problems of modern flowering which for long have been the special study of the present writer.

The final postulate is that all known vascular plants are descendants from the same or similar ancestral stocks, have essentially similar physical constitution, and that in consequence a uniform system of morphological concepts should be applicable to all. Ferns and Angiosperms are thus admitted to comparable ancestral history and, under the mantle of the former, the Caytoniales are at last ready to enter the arena to handle the problems of angiospermy, though sharing with their guardian defects of subjectiveness.

Their qualifications for the interpretation of modern angiospermy are not, however, presented until a further essential for a morphological system is explained, namely that it must be applicable to all facts of structure and development. The gamut of carpel ontogeny, mature form and structure, carpellary vasculature, styles and stigmas, transmitting tissue for pollen tubes, abnormalities, the results of experimentation, ovule form and placentation, and fossil records are admitted as evidence in the reading of carpellary history. The modern androecium and perianth are, however, excluded from enquiry, despite an earlier statement that "we must consider the floral organs as interdependent parts of a whole living plant," and the fact that they antedate the carpels in ontogeny on a common receptacle. How fatal is this omission to the cause of the Caytoniales will later be indicated.

At this point Dr Thomas comes to grips with the problems of angiospermy and firstly with the questions as to how, when and why did the stigma arise.

He disposes of the conception of an ancient glandular tip of an expanded fertile leaf as raising questions which cannot now be answered; he rejects the inrolling of leaf margins before the evolution of pollination through the medium of a stigma as involving a mutation unfavourable to the race, and considers it unlikely that the appearance of the stigma and the inrolling of the leaf would occur simultaneously in a single mutation.

Having already stated that all known facts of structure and development are significant, he now turns to facts of development of stigmatic styles as recorded by the writer in his "Theory of Scitaminean Flowering." It is there shown that the Scitaminean styles are potential stamens, and that no valid opinion on the nature of the inferior ovary in this affinity can be reached without consideration of the flower as a whole. In the absence of detailed descriptions of the stigmatic surfaces of the styles Dr Thomas states that the writer's view of androecial origin for the styles "appears to have little objective foundation," and "cannot now be usefully discussed," despite the fact that it offers the first "objective" statement on stylar origin.

Dr Thomas's statement of the interpretation offered for the Scitaminean flower is next in error, for he quotes the writer as regarding "the primitive flower" as possessing only vegetative emergences, stamens, and stalked ovules borne on the crater-like receptacle.

At no point has the writer offered a view on the organisation of "the primitive flower," nor has he conceived as primitive a crater-like receptacle as above described. He has, however, suggested from intimate study of Scitaminean flowering that in the immediate ancestry of the *Scitamineae* the flower was as depicted, and to this suggestion he is inclined to adhere.

Dr Thomas now turns to the nature of the stigma of living plants. He quotes from the observations of Capus, Guéguen and Juel on the presence of glandulation in the ovaries of certain living Angiosperms and on the path of pollen tubes, and feels justified, without any ontogenetic knowledge of the styles or ovaries selected, in regarding the stigmatic surface as a specialised part of the inner wall of the ovary commencing basally and extending upwards. He declines to accept the view that stigmatism may declare itself first at the apex of a style, although it is manifest in many affinities that stigmatism is thus accomplished. The truth may partly be covered by the statement that there is no such organ as a stigma, that stigmatism is of varied locus, extent, inception and duration, that it may occur

externally to the ovary, and that it expresses a state of tissue in some way associated with limitation of growth.

There is no evidence for the conclusions of Dr Thomas that "the stigma originated on the ventral side of the carpel, that subsequent development of the style, or of the ovary wall has gradually carried it upwards, and that it is a structure appearing to be composed of two adjacent lobes or parts, the contiguous facies of which are lined by glandular hairs and continuous with transmitting strands below."

The discussion on the nature and origin of the stigma is not, however, completed, and the Caytoniales cannot play their part without distraction until the carpels of *Drimys Winteri*, *Magnolia*, and others have borne testimony to the dual nature of the stigma: for it is now apparent that since each cupule-like envelope of the Caytoniales is to provide a stigma and that fusion of such envelopes in pairs is to be assumed in the making of a carpel, the stigma of this fusion-product must be dual. Dr Thomas's statement on *Drimys* may suffice to illustrate the approach. An advanced carpel is figured, its undivided and U-shaped terminal stigma is described as a double lateral structure, and the fact that glandulation is not restricted to a clearly circumscribed area, but extends to the adjacent placentae is held in some way to furnish support for his contentions.

A suggested origin of the stigma is now reached, and, on the matters analysed above, and on them alone, it is stated that "fortunately we are now able to outline a possible history of the evolution of the carpel which would co-ordinate the varied facts of structure and combine them with what we know of fossil seed plants. One may call it a history rather than a phantasy because it commences with a group of plants which actually existed, and is based on the comparison of the plants of successive ages."

The Caytoniales are the group of plants referred to. Their qualifications as interpreters of modern Angiosperms still remain—that they possess cupule-like envelopes for their seeds, dissociated in any strict sense from microspore-bearing organs, and that their affinity is unknown.

The final phase in Dr Thomas's statement is now reached. It is offered as a legitimate assumption that cupule-like bodies have fused in pairs, so that we should have a structure "which is in all essentials an angiospermous carpel." The original ovule-bearing stalks become adjacent placentae, the recurved papillate tips of the fused cupule-like envelopes form a pair of lateral stigmatic surfaces, and the whole ovuliferous structure is imagined reduced, so that the equivalent of a

modern gynoecium may be provided. In so doing Dr Thomas has thrown his principles of objective reasoning to the winds and draws his study of modern angiospermy to a conclusion satisfactory to himself. Such minor difficulties as the possible re-entry of the modern androecium and perianth (which, together with the major portion of his essentials for a morphological system, has been forgotten in his discussion) remain for later disposal.

In reflecting on the approach to angiospermy herein revealed the writer would point a moral from his initial quotation. With others he will welcome the testimony of authentic Angiosperms from the rocks, and until this testimony is provided the Caytoniales, as at present known, will remain—the Caytoniales.

And lastly, Dr Thomas has requested a statement on the foundations of the beliefs of others. In so far as the writer is concerned a statement is gladly offered. He believes that by the study of living Angiosperms the essential problems of angiospermy may be exposed. The foundation of this belief is observation from the floras of many lands. By such observation he has reached tentative views on some of the salient problems of modern angiospermy, and they are as follows:

1. That in all species which he has examined the floral receptacle is potentially heterosporous.
2. That microsporangia and megasporangia alone are essential products of the receptacle.
3. That anthers and nucelli are these essential products.
4. That curtailment of the receptacle is widespread and progressive both ontogenetically and racially.
5. That, as in leaf formation, this curtailment leads to emergence of the receptacle.
6. That emergence is compensation for failure of apical growth.
7. That by such emergence anthers and nucelli are raised from the receptacle, and stamen filaments and funicles are provided.
8. That with limitation of growth of nucelli integuments likewise express diverted growth beneath the nucelli.
9. That when, in ontogeny, the limits of apical growth of the receptacle are imminent, while microsporangium formation is slowly progressive, successive emergences are formed. Each emergence temporarily satisfies the needs of growth, the microsporangium is "broken" into minor tracts, and numerous stamens result.
10. That when limitation of apical growth is more rapid fragmentation of the microsporangium is less marked. Emergences are

fewer, individually they are relatively massive, and the resultant stamens are few and relatively large.

11. That sterilisation of the upper zones of microsporangium commonly occurs.

12. That emergence of the receptacle by toral growth may be added to discrete emergence at any point in ontogeny at which the latter no longer fulfils the requirements of growth.

13. That such toral growth determines the final form of the flower according to its incidence and persistency.

14. That when stamen formation is still in progress and toral growth is added, both perianth and androecium may be raised on the torus so that some members of the androecium are on or near the toral rim.

15. That these upper stamens are commonly sterilised, constitute the styles, and later become glandular.

16. That the basin formed by the torus is purely receptacular, is throughout potentially megasporangial, and that as it is extended it bears emergent nucelli varying in both number and size, as is the case with the anthers and their filaments.

17. That the original diameter of the basin limits expansion, that in-growth occurs in varied degree beneath the styles, and that placenta¹ are thus created.

18. That placenta¹ are the tracts of the basin best nourished and on which ovulation is most readily maintained in what is now known as inferior ovary.

On this view there are no carpels involved in the organisation of an inferior ovary, their existence need not be considered a necessary prelude to ovulation or as intervening between microsporangia and megasporangia, and there is no sinking of bodies of any form whatsoever into the receptacle. The state of angiospermy is fulfilled, and limitation of apical growth accompanied by toral growth dominates the moulding of the flower. There is no call to consider this form of angiospermy inexplicable without reference to ancient fossils nor to stretch their scanty evidence into a phantasy and to call the latter a history. The writer has already furnished some short chapters in the events from a single living affinity, and will extend his statement to ovulation at an early date¹.

It is, however, apparent that until the facts of gynoecial ontogeny for some family with superior ovaries are made known many will

¹ Publications of The Hartley Botanical Laboratories, No. 12, *On Placentation and Ovulation*. A preliminary statement based on ontogenetic study.

rightly hesitate to share the writer's views. He is therefore providing facts of observation on the Ranunculaceae supporting the view that the early course of ontogeny is as above presented under headings 1 to 13, that in the formation of follicles sterilisation of stamens is involved; that by toral growth from beneath the arrested apex of the receptacle the sterilised emergences are variously united; that as the torus persists in growth it supplies the receptacular surface on which ovulation is expressed as in the inferior ovary; and that the distinction between superior and inferior ovary is merely one of description.

The matter is considered likewise in the formation of achenes, and again carpels as historic documents in the story of angiospermy are considered unnecessary.

The writer confesses to a return to a view of flowering not far removed from that of Schleiden of a hundred years ago, though it may be expressed in different terms.

In issuing these pages in response to what is considered improper criticism and approach to a complex problem he has been forced to anticipate reasoned statements on observations made, and believes that men of science will await with patience the story of the living Angiosperms.

SUBSTITUTION STAINING WITH FREE DYE ACIDS AND DYE BASES

By R. C. McLEAN

THE acid dyes eosin and erythrosin in their usual states are sodium salts of dibromodichlorofluorescein and tetraiodofluorescein respectively. They are soluble in water but sparingly or not soluble in most organic solvents. The corresponding free acids are, however, soluble in ether or xylol and can thus be separated from an aqueous solution of the dyes.

This simple piece of dye chemistry has, however, an importance in microtechnique which does not appear to have found its way into the literature of histological practice.

Procedure. Make a 1 per cent. aqueous solution of either eosin or erythrosin. For every gram of erythrosin in solution add 2.5 c.c. of a 10 per cent. solution of hydrochloric acid of sp. gr. 1.16. For every gram of eosin add 3.5 c.c. of the same acid. Precipitation is immediate. The simplest procedure is then to allow the deposit to settle for 24 hours, pipette off the clear liquid (the slight excess of acid assists sedimentation), add pure xylol to the residue at the rate of about 200 c.c. for each gram of dye used and mix by gentle shaking or by stirring with a glass rod. Avoid strong shaking in the case of erythrosin, or a viscous emulsion may form. Pour into a separating funnel and continue gentle agitation as long as any solution is taking place, then allow to settle and run off the xylol extract, which will be practically colourless.

The dye acids are not highly soluble, so that if a stronger solution is desired the original precipitate should be filtered on to asbestos (not paper) in a Gooch crucible, air dried and finally shaken up with dry xylol in a mechanical shaker.

From the colourless solution the colour is again produced by the addition of alkalis or by an electronegative adsorbent such as cellulose, with which an ionic exchange can be effected. This may be seen by dipping a piece of filter paper into it.

Sections of plant tissue are first stained with methyl violet or methylene blue, washed as usual in methylated spirit and absolute alcohol to remove the stain from the cellulose walls, then transferred to xylol. From this they are placed in xylol eosin, when the red

Substitution Staining with Free Dye Acids and Dye Bases 317

colour develops immediately. Prolonged staining is of no advantage, the sections should be at once transferred to xylol balsam or some other resin, as permanent mounts.

Counterstaining is thus made a perfectly mechanical procedure, coming at the most convenient point of the operations, immediately before mounting, and all difficulties of differentiation are avoided.

Eosin gives a more orange tint than erythrosin, but otherwise their effects are the same. The colour formation is also a test reaction for cellulose in the wall, and it is interesting to observe that, while lignified tissue as a whole is unstained, the protoxylem vessels and those in process of formation at the inner cambial boundary show a half-red tint, while metaxylem and sclerenchyma are entirely colourless, unless counterstained. Cork and pith cells are also uncoloured, but in the latter tissue the intercellular trigones are strongly stained. Cytoplasm and plastids are lightly coloured or not at all, but nuclei are stained.

The free base of methylene blue (Nile blue is better) may be used in a similar way. Add 2.5 c.c. of 10 per cent. sodium hydroxide for each gram of dye in solution. Precipitation takes several hours. The xylol solution of the base has a slight purplish red tinge, but sections of plant tissues placed in it have their lignified membranes immediately stained bright blue.

It is not possible to combine the xylol eosin with this stain in one solution, but they may be used successively.

Place the sections first for a few moments in methylene blue xylol and then transfer directly to eosin xylol, when a perfect double stain is automatically achieved.

The process was originally suggested, though fallaciously, as a test for alkali in tissues. It avoids so simply the difficulties and uncertainties of other staining processes that it deserves further study.

REFERENCES

- MOLISCH. *Mikrochemie der Pflanzen*, 2nd ed., p. 27. 1921.
HOF. Untersuchungen über die Topik der Alkaliverteilung in pflanzlichen Geweben. *Bot. Cent.* **83**, 279. 1900.

REVIEWS

Researches on Fungi. Vol. v. By A. H. R. BULLER: London: Longmans, Green and Co., 1933. 9.4 in. x 6.2 in. Pp. xiii + 416 with 174 figs. 25s. net.

Prof. Buller's *Researches* need no introduction. They rank among the foremost in mycology. Indeed, with the publication of each successive volume, we have learnt to expect issues of greater import and finer execution, and in these latest we are in no wise disappointed. This fifth volume proceeds from general topics of the mycelium by many diversions to the elucidation of one of the most specialised mechanisms of the Gasteromycetes, and for its discoveries, its conclusions and its clear and ingenious exposition we consider it to be the best. It is modelled on its predecessors; a generous summary and a wealth of illustration supply the essence, and one is guided through the details of an explicit text by a copious index.

The first part of the book, which continues vol. iv, deals with the subjects of hyphal fusions and protoplasmic streaming and will appeal particularly to the physiologist because it adds so much to our knowledge of the construction of the mycelium and of the interchange of materials within the hyphae. The various ways of fusing are described to the effect that all are "end-to-end" fusions representing "action at a distance" of one hypha on another. And in disproving Burgeff's explanation of this phenomenon (for the clear presentation of which we are most indebted) Buller points out that so ordinary an occurrence leads on consideration to a problem of the first magnitude, at present insoluble and demanding, perhaps, the concept of vital radiation. Experiments in hybridisation, by observation of hyphal fusions, give a check on the work of the systematist and support his conclusions, especially in the case of the Hymenomycetes, where species-making has been deemed a trivial affair. This should be a rich field for research.

Protoplasmic streaming in hyphae (at rates up to $55\ \mu$ per sec.) occurs in many fungi, though its significance can yet hardly be appreciated. "In a mycelium of *Pyronema confluens* protoplasm was seen streaming through 161 successive cells... The length of the stream exceeded 1.6 cm." There must be passage-ways through the septa, and Buller now demonstrates beyond dispute that the septa of all Eumycetes are persistently perforate in the centre, unlike those of Phycomycetes which are imperforate when fully developed and serve not for the transmission of protoplasm but for the retention of it. We are presented with the novel picture of the mycelium of a hymenomycete, diploidised perhaps by means of the septa and further compounded by fusions with other mycelia, through the hyphae of all of which, independently of their origin, protoplasm is propelled to a common end by the motive power of vacuolation. The fruit-body is inflated by this same force, which drives also the protoplasmic reserves from the mycelium up to the growing-points and ultimately into the extremities to be discharged in the spores.

There are important digressions too on clamps and septa in general, on "Woronin bodies," on rates of nuclear division and apical growth, on the effect of wounding hyphae and on regeneration. But few points we would contest. The toughness of the fruit body of *Marasmius oreades* (p. 2) is surely due not to the numerous hyphal fusions but to the slight thickening of the hyphal walls, the interweaving of the hyphae and their relatively slight inflation. Further investigation is needed to show how general hyphal fusions may be in fruit bodies, especially among polypores and telephores. A closer analysis of the mycelium of *Pleurage curvicolle*, for instance, might have revealed a structural

unit corresponding with the physiological, e.g. a peg from each cell. On p. 66, line 3, "1900" is surely a mistake. The biological advantages attributed to the construction of septa and clamps are clearly effects, not causes, and we are still left in the dark concerning their origin. We gladly read that clamps are only analogous with ascogenous hooks, the supposed homology having always appeared artificial. But we should like to know the exact difference between vegetative (nutritive) fusions and sexual fusions.

The second part is in three chapters, each devoted to particular fungi. The first is a review of the *Sporobolomycetes* with a special account of *S. roseus*. Buller concludes that they are basidiomycetes. The spores have precisely the shape and mode of discharge of true basidiospores, and these properties, the author maintains, are the criteria for the homology of basidiospores, inasmuch as they are found in no other kind of spore: and, in view of the comparative evidence adduced from the smut basidium in the succeeding chapter, there is but the alternative to create a new phylum and beg the question. The author independently corroborates Guillermond's observations that the cells and spores are uninucleate, no binucleate phase having been discovered: and such anomalies as successive spore-production and branched sterigmata are readily accounted for. Those who consider fungi to be the subaerial expression of marine filamentous phytobenthon will welcome this discovery of a family of basidiomycetous yeasts; their theory postulates that yeasts shall be the last term in reduction of any fungus phylum. But is it proven that the *Sporobolomycetes* are not the basidiospores of smuts? Fig. 90 displays the futile simplicity of these little organisms which, expending all their energy in absorbing food, blindly get rid of it by an elaborate mechanism, in delicate spores, after a few hours and within the compass of a few microns. Can they be primitive?

The second chapter will probably be most widely read. It is the best account we have of the common class-fungus *Tilletia tritici*: accuracy in observation is combined with fullness and excellence of illustration, so that there seems nothing to be desired. According to his conception of the basidiospore, the author interprets anew the basidial apparatus of the smuts and introduces a revised terminology, aptly supported by a limited but effective comparison of the basidial apparatus in three other genera. It is greatly to be preferred to the Brefeldian terminology, current in text-books, although there is the regrettable anomaly—that "secondary sterigmata" should produce "primary basidiospores": such a term as "prosterigmata" might have been preferred for the "primary sterigmata." Characteristic of the thoroughness of Buller's methods are the descriptions of six variations in the development of the basidium and basidiospores, one only of which is really normal and that is here described for the first time. The septation of the promycelium is described in detail and many facts are gathered of the nuclear behaviour in smuts, of the water-absorbing power of the mycelium of *T. tritici*, of the close relation between germination of the basidiospores and drying, and of the chlamydospores and "ventilation," and of the negative hydrotropism of the promycelium, which is an astonishing variety of water-requirements in an endophytic fungus. Although *T. tritici* is highly specialised and reduced, it shoots away its spores with greater violence than any other basidiomycete. There appears a slip on p. 33 (three lines from the foot of the text): "secondary" should read "primary."

The final chapter is a history and description of the gasteromycetous genus, *Sphaerobolus*. The researches of Leva Walker are corroborated and extended, and we have now a thorough knowledge of the structure, working and kinetics of this marvel of hyphal engineering (though it still remains to be discovered exactly how it is developed from interweaving hyphae). The fruit-body is a miniature mortar with "the largest known fungus projectile" and it is "the largest, most powerful and the loudest of all fungus guns": little wonder it attracted the author's curiosity, especially when it hit the ceiling in his office. After describing minutely the structure, Buller shows how practically every feature of the fruit-body, macroscopic and microscopic, is concerned in the

perfection of the mechanism. The motive force is the rapid eversion of the inner peridial membrane (in 1/1500th sec.) and, though paralleled by the Geasters, in power and effect it is unique: it is, nevertheless, a hydrostatic mechanism as in all methods of violent discharge in fungi. *S. stellatus*, which has been known to science for 200 years, is reckoned a common species, yet Buller is the first to have shown that it is a coprophilous fungus of the most advanced kind, comparable with *Dasyobolus* and *Pilobolus*. The chapter closes, as one would never have foreseen, with an instinctive comparison between the two main types of coprophilous fungi. And, as befalls all upon which his wand alights in his love of mycology, *S. stellatus* becomes the best known of all gasteromycetes. We suspect the family of some phalloid origin, chiefly on account of the form of the basidia and spores, the gelatinisation of the peridium, and the glebal cavities found in *S. iowensis*. On p. 321 (one line from the foot of the text) for "osmotic pressure" it seems we should read "turgor pressure."

It is clear that these *Researches* are indispensable to the mycologist, be he physiologist, morphologist or systematist. And if they are a little laboured and in parts overwrought so as to be void of misunderstanding, there is a finality about them which we would have at any cost.

E. J. H. CORNER.

Introduction to Cytology. By L. W. SHARP. 3rd edition. McGraw Hill Co., 1934. 9 in. \times 5½ in. Pp. 567 + xiv. 230 figs. in text. 30s.

The earlier editions of Sharp's *Introduction to Cytology* have been widely used by students in the universities of the United States and this country. They provided in a limited space everything that was deemed to be academically essential in the subject. They were compiled by a respected teacher. Their point of view was morphological and static, and could readily be assimilated by the student accustomed to other morphological and static branches of biology. The new edition, in spite of the difficulties created by recent innovations in the subject, preserves this point of view unchanged and its academic adaptation is perhaps even more skilfully carried out. It is again cautious, careful and complete. But the text is shortened and is now straightforward and unencumbered for the elementary student. The bibliography is lengthened and of the esoteric references, now confined to footnotes, none have been omitted that might be of use in higher degree theses. In the index, authors are more numerous than organisms, an indication of the widespread desire to emphasise the bibliographic aspects of biological research.

What will the student find? By the third page he will already be armed with a battery of words with which to confront his examiners. He will know that a living organism is a "protoplasmic system." Its transparent contents are always "hyaline," a network in a fixed nucleus is a "reticulum." He may later, if he reads Belar, learn to know it as an "artefact." But this is one of the words that has not got into the index. This is enough for a beginning; but words are very important in this study (Denn eben wo Begriffe fehlen da stellt zur rechten Zeit ein Wort sich ein). Later the *Introduction to Cytology* will tell the student what an atelomitic isobrachial heteropycnotic idiochromosome is. It will tell him how an undefinable chromonema winds its way through an illimitable matrix. Why there is no glossary we shall learn later.

Having learnt the passwords the adventurer will feel free to enter the realm of constructive study. If he is used to the reasoning of chemistry and physics that is his misfortune. For he must now be inducted into the less rigorous methods of an inexact science. Consistency here is less important and definition is always of the *ignotum per ignotius* type. The student must not be puzzled to find the modern trivalent of three chromosomes arising amidst the ruins of ancient tetrads of two chromosomes, for the Greek unit is half the Latin one and the roots are interchangeable. But if he is not familiar with the philosophic

system concerned he will be puzzled to learn that in this branch of biology all antecedent conditions are taken to be sufficient causes until the contrary is proved. Thus "synapsis is not solely a matter of gene homology but is influenced by some condition pervading the nucleus or cell generally." If the student is not too much taken aback by this he will proceed to learn something quite new about homology itself (in inverted commas): the pairing chromosomes "are 'homologous' not only because of their ultimate common origin but also in the sense that they perform similar rôles in the life of the organism" (p. 272). In the circumstances it is too much to ask for a glossary.

This book is therefore not merely an exposition of biological facts. It is a demonstration of a biological method now much in favour. But the method leads to trouble. It leads to a lack of rigour in observation and of relevance in arrangement. Now cytology is after all becoming a science, and observations must be constantly compared and their inconsistencies resolved. This particularly shows itself in the inductive-deductive aspects of the study concerned with mechanical and genetical theory. The theories of mechanics are here divided between chapters on chromosome morphology and crossing-over. The "kinetic bodies" observed with one fixation are said to lie in "insertion regions" found with another. They "take the lead" in anaphasic movement (p. 116) and one might almost suppose that the rest of the chromosomes follow willingly. Sharp's illustration of what are apparently meant to be these bodies shows them quadruple at metaphase (Fig. 63). But all facts are free and equal. On the same page Navashin's figure shows them double. The student may take his choice. Or he may prefer to follow the author's example and remain ambiguous on matters where authorities differ. Then "tetrads" at meiosis are said to "take up their metaphasic positions with their attachment regions in the equator" (p. 262). Strange to say, this never happens at meiosis. They lie as near to the equator as the tropics of Cancer and Capricorn. But it would be true of ordinary mitosis and the difference provides one of the critical distinctions between the two processes. No distinction of effect is made between primary and secondary pairing at meiosis, although the one determines segregation and the other does not even influence it. Small wonder then that the author finds it interesting "to speculate upon the possible relation between the synaptic 'attraction' and the later 'repulsion'" (note the inverted commas) and no wonder at all that he finds that "such speculations have as yet little of a definite nature to support them." Presumably this part of the book was written in 1931.

The illustrations of mitosis and meiosis are consistent with the author's timidity in dealing with the part of the subject in which he himself has specialised. There is one figure of genuine historical interest—a drawing of chromosomes by Hofmeister in 1848. But there are too many others which are neither very old nor very good. No one can complain at the author reproducing his own figures of mitosis (Fig. 73) but the writers from whose papers Figs. 153, 155a and 191 are taken might with kindness have been spared being confronted with the results of their earlier work.

It is in considering its relationship with genetics that the writer on cytology has his obvious opportunity of synthesis, and one is naturally interested, remembering his chapter in earlier editions on "Weismannism and Other Theories," to see what Sharp has made of it. There is a considerable account of data of genetics. Remarks on breeding experiments are found in chapters 16, 17, 19, 23 and 25, and short passages in praise of the chromosome theory and genetic methods encourage the reader and bring chapters 9, 18, 19, 23 and 26 to a close. But what the student needs is not just a statement of the principles of genetics but an application of them as a working method in cytological research and as a basis for the arrangement of its data. This he will seek in vain. No attempt is made to show how the chromosomes of an organism genetically control their own behaviour in every phase of life. No attempt is made to present the critical evidence for or against any theory of crossing-over. On the other

hand, conclusions which, if they were true, would have the utmost theoretical significance are casually stated without any consciousness of their gravity. For example non-homologous association "usually disappears before diakinesis" (p. 276). There is no evidence of its ever being retained at diakinesis. The consequence of this method is that no conclusions can be arrived at with regard to the genetic interpretation of chromosome behaviour in hybrids or polyploids, or in apomictic or other aberrant species—the word species is not even indexed. Equivocation is everywhere. On this foundation any attempt to use chromosome studies as a tool for genetical or taxonomic research is out of the question.

There are many who delight in the study of facts compiled with impartiality and uttered with authority. There are some who find that a logical nexus is a hindrance rather than a help to this study. To such this book can be recommended. For amongst them it cannot fail to be useful: it will save the teacher teaching and the student thinking.

C. D. DARLINGTON.

Manometric Methods as applied to the measurement of cell respiration and other processes. By MALCOLM DIXON, M.A., Ph.D., Sc.D., with a foreword by Sir F. G. HOPKINS, P.R.S. Cambridge University Press, 1934. 7½ in. × 5 in. Pp. ix + 119. Price 5s.

Manometric methods of measuring gas exchanges were first developed on modern lines twenty years ago, and their utility in physiological research has been amply demonstrated. The reacting material, immersed in a small volume of fluid, is placed in a closed system, and either amounts or rates of gas exchanges may be accurately measured on a delicate manometer; similar vessels serve as controls. Various very ingenious modifications of the apparatus permit of the measurement of both oxygen uptake and carbon dioxide output on the same piece of tissue.

In this laboratory handbook Dr Dixon passes on the benefit of twelve years' experience in the design and use of the chief types of manometer available. He gives an account of the theory involved, and, what is perhaps of most use to the isolated worker, adds numerous details of technique which should minimise errors, and save much time. Those relating to the rate of shaking, and the limiting thickness of tissue are especially valuable. Care must be taken, however, in applying the formula of p. 50 in determining this limiting thickness d' of the tissue used. The rate of respiration at the cut surface of some plant tissues is probably much greater than that of the internal cells; moreover quite high concentrations of oxygen may allow of fermentation. The formula does not take such facts into account, and probably only an empirical determination of the effect of tissue thickness on the r.g. will be satisfactory in estimating d' . Manometric apparatus gives results quickly and is almost fatally easy to use, and it is all the more important that the theory and methods should be most carefully examined. Dr Dixon has carried out this task very thoroughly. It is his critical attitude, and the bringing together of the various scattered references which give the book its main value.

The use of manometry in plant physiology began with Warburg's well-known researches on photosynthesis and respiration in *Chlorella*. In the fifteen years since this was published, only a few workers (e.g. Genevois, Emerson, Arnold) have used his methods. The contrast with the rapid advance and numerous improvements in the methods described here in the field of animal physiology is most startling. It is typical of the unfortunate lack of correlation or even of sympathy between plant and animal physiologists, that no mention of a plant other than yeast or bacteria, occurs in Dr Dixon's book, although the utility of the methods along various other lines is pointed out.

Both the Warburg manometer and the differential Barcroft have been used in work on Photosynthesis. The difficulties are in supplying the plant with carbon dioxide, and in controlling the illumination and temperature. Warburg describes his own solution of these problems, but probably further research would lead to improvements.

Manometry may also be applied to the study of the respiration of the excised tissue of the higher plants, although few of them will withstand the extensive wounding or even maceration which is the recognised procedure in dealing with liver, muscle or brain tissue, and the effects of which are too often ignored by biochemists. It would seem that only by manometric methods will it be possible to bring our knowledge of plant respiration into line with the great and growing body of facts now collected for yeast and animal tissue. There is no doubt but that Dr Dixon's book will be of service in bringing about a wider and more intelligent use of the methods he describes.

J. S. TURNER.

School Botany. By MACGREGOR SKENE. 2nd edition. Oxford University Press, 1934. 7½ in. × 5 in. Pp. vi+278. Price 3s. 6d.

The early call for a new edition of this successful little book has enabled it to be extended and improved in detail, and one may sincerely hope with the author that its usefulness may thus be still further increased. It professes to cover the field of matriculation botany, introducing its readers to the structure and activities of plants mainly in terms of the angiosperm. In the new edition a chapter on other kinds of plants has been added and deals very briefly with conifers, ferns, mosses, algae, fungi and lichens.

Although the bulk of the book deals with its matter in a fairly orthodox manner, there is a certain freshness of treatment that makes it agreeable reading even to those who have read elementary botany books before. This will probably not surprise readers who are already familiar with Dr Skene's earlier publications. Most botanical handbooks tell you that photosynthesis was discovered by Joseph Priestley with the help of a mouse, a sprig of mint and a bell jar. Not many tell you that Priestley's thoughts were set to work in this direction by an entirely false clue given him by the landlord of an inn at Harwich. (Incidentally not even Dr Skene tells you of the difficulty Priestley had in repeating his famous experiment.) In this special example it may perhaps be thought that the author goes rather far. Is it wise to point out to the learner at such an early stage the undeniable fact that many of the most fruitful discoveries have been arrived at more by lucky chance than by unimpeachable reasoning? At least it may be pleaded that this cruel "debunking" should not be extended to living workers.

Among much that is excellent and stimulating one major point of treatment seems curiously outmoded. Thus, on p. 105, "Plants often store up food for future use"; on p. 93, "Here is a new kind of leaf, very different in form from a foliage leaf, and doing quite different work"; there are also numerous chapter headings such as "The Flower and its Work." It may be argued, of course, that these phrases are literally correct, nevertheless we do not usually say that a mixed solution of salt and silver nitrate stores silver chloride; nor that iron and nickel do different work because their properties are not the same. The fact is that these words are liable to be misunderstood and never more than when describing plants to the human young. Rightly handled, a biological training may be the best specific against anthropomorphism, but there are obvious pitfalls.

The illustrations by Miss Ruth Weston are line drawings that for the most part can safely be shown to pupils as examples of what should be attempted, but we devoutly hope that none will adopt the method of flower drawing

applied to the buttercup and rose in Figs. 36 and 52. Surely it is time that this venerable error was interred. The opposite extreme in Figs. 38 and 39 (poplar and plantain) is not really much more informative, but a happy inconsistency has led to better results with the daffodil, primrose and some others.

A feature of this reissue that catches the eye is the abandonment by the publishers of their familiar blue and gold cover in which the first edition appeared and its substitution by a pleasant green ornamented with a leaf of *Aconitum napellus*.

W. O. JAMES.

THE NEW PHYTOLOGIST

Vol. XXXIII, No. 5

14 December, 1934

POLLEN ANALYSIS. AN OUTLINE OF THE PROBLEMS AND POTENTIALITIES OF THE METHOD

PART II. GENERAL APPLICATIONS OF POLLEN ANALYSIS

By H. GODWIN

Botany School, Cambridge

(With 10 figures in the text)

CONTENTS

	PAGE
Introduction	325
Local influences and regional parallelism	326
(a) Succession	326
(b) Soil conditions	329
(c) Elevation	331
(d) Regional parallelism	333
Correlation of forest history with other time indices	337
(a) Geological	338
(b) Climatic	342
(c) Archaeological	346
Applications of pollen analysis to the British Isles	351
References	356

INTRODUCTION

WE have so far been primarily concerned with pollen analysis data from single samples, or from a single vertical series of samples. In practice, however, the method always involves comparison of the analyses of many vertical series distributed over a region of greater or less extent, and it is the problems and principles of such wider comparisons which are discussed in the following section of the paper.

It is important to realise a twofold aspect of the general application of the methods of pollen analysis. *In the first place* regional

pollen analyses may be employed to construct a key to the vegetational history. The sequence of forests upon the area will be clear in the parallel drifts of the analyses from the various sites investigated. In consequence of this it may be possible to draw conclusions as to the sequence of former climatic conditions upon the area, though it must be remembered that within each present-day tree "species" is a widely ranging series of ecotypes with differing ecological requirements.

In the second place regional pollen analyses may be used as an arbitrary chronological scale, either for post-Glacial or inter-Glacial times. Such a scale is of the greatest importance not only for a study of vegetational history, but also for dating the recent spread and development of animals, of races of men and their specific cultures, and for dating geological events, such as coastal movements. For the purpose of such an arbitrary yard-stick to past chronology it is not necessary that the pollen analyses should reflect forest composition with absolute truthfulness, though it is necessary to be assured of the synchronism of the parallel drifts shown in the various vertical series of analyses. In general this synchronism is based on widespread climatic change, and in so far as time is needed for the spread of forests and in so far as different topography, elevation, distance from the sea, latitude and other factors also affect forest distribution, the simple effects of climatic change will tend to be obscured or at least complicated.

The use of pollen analyses in making an arbitrary time scale is especially important in the post-Glacial period, since the time has been too short for the evolution of suitable animal zone fossils, and since human remains or artefacts are far from sufficiently frequent to serve this purpose. Pollen analysis shows to particular advantage, since the pollen grains are so extremely abundant as to become incorporated in all types of growing deposits, and since they are well represented in such fresh-water deposits as peats which are not only very widespread but which tend to be particularly poor both in cultural and animal remains.

LOCAL INFLUENCES AND REGIONAL PARALLELISM

(a) *Succession*

The influence of local factors upon the pollen rain incorporated into the peat of a growing bog has long been recognised in pollen-analysis investigations. In the case of the development of ombrogen-

ous *hochmoor* we have already noted that the trees growing on the bog surface and those in the wet marginal woods yield recognisable components to the total pollen rain. It is not likely that these components will be the same throughout development of the bog, which in many cases begins with the neutral or alkaline peats of a local depression, passing through phases of active growth of acid *Sphagnum* peat, and finally coming to a condition of arrest or erosion (see p. 344).

Even more subject to the local influences of bog development are the deposits of topogenous moors such as those now forming in the Norfolk broads and those which have built up the peats of the East Anglian fenland basin. The diagram (Fig. 10) shows the pollen analyses through a two-foot peat bed in the fen beds at St Germans, near King's Lynn (47). It lies between two beds of semi-marine clay and its upper surface is about three feet below present mean sea-level. The key to the history of the region is given best by the non-tree pollen and fern spores. It will be seen that the lowest samples B₃ and B₄ are marked by considerable amounts of a type of pollen found in the Chenopodiaceae and the Alsineae (Caryophyllaceae). This is interpretable as due to the former presence of salt marshes in the neighbourhood, since plants of these families are probably more abundant there than in any other natural plant community in this country. After this in succession come maxima (1) of pollen of Gramineae and Compositae, (2) of Salicoid pollen and fern spores, (3) of Salicoid pollen with a large proportion of *Fraxinus*, (4) of the total tree pollen, (5) again of fern spores and (6) lastly of Chenopodiaceae-Alsineae pollen again. These maxima suggest the vegetational sequence indicated on the right of the diagram, namely through young to mature salt marsh, with abundant Gramineae, alder-willow scrub with abundant fern undergrowth, young ash-oak wood progressively killing out the earlier scrub until finally pure oak wood is indicated by the high absolute value of the pollen and the Filicales maximum. The final rise suggests salt-marsh conditions returning again, a suggestion borne out by analyses of Foraminifera (58), which show brackish water conditions during deposition of the clay overlying the peat. The tree pollen also reflects the same succession, especially the very marked increase in *Quercus* pollen and corresponding decrease in *Alnus*. The same inversion in the relative importance of these trees is frequently shown from the base upwards through the fen beds and progressive building up of peat above the water table by the natural "reaction" of the prisere. *Betula pubescens* may also be a component of the

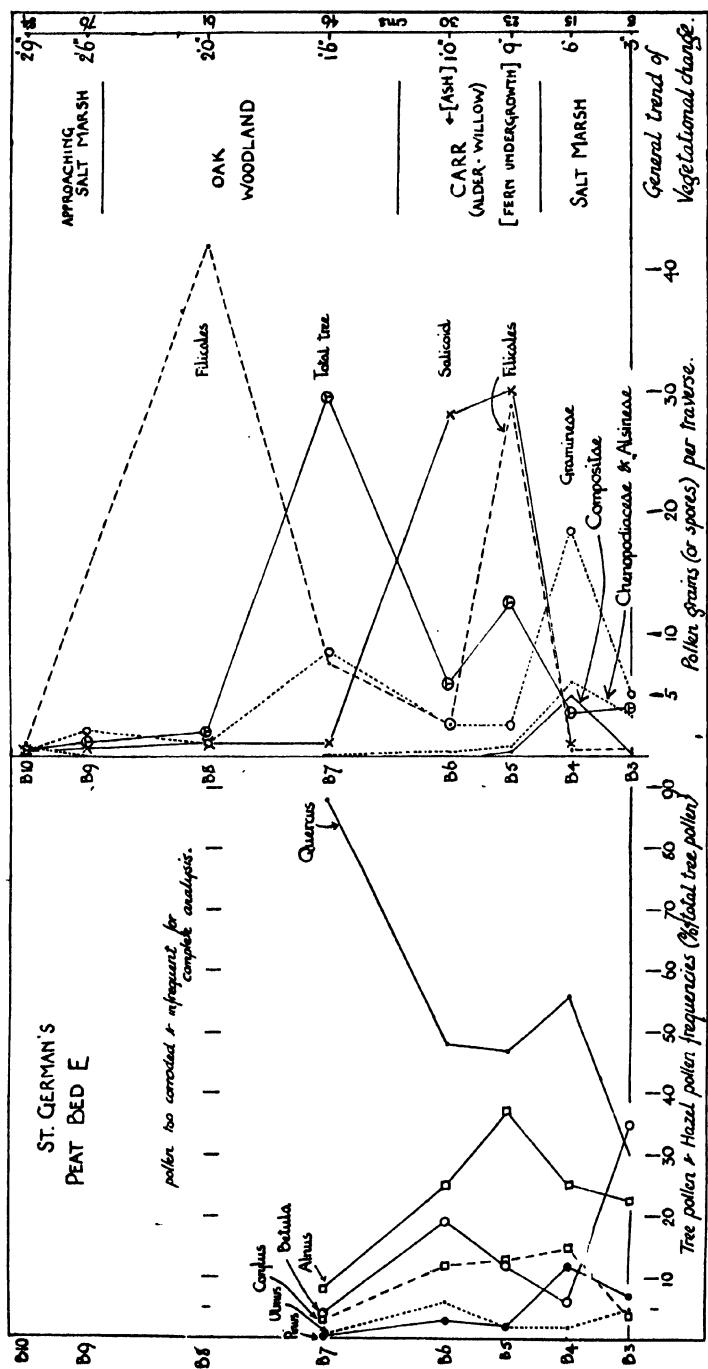


Fig. 10. Pollen analyses in sequence through a two-inch peat bed intercalated between semi-marine clays at St Germans, near King's Lynn. The left of the diagram shows tree pollen and the right non-tree pollen and spores. These diagrams are interpreted as indicating the vegetational succession set out on the extreme right of the figure. (Reproduced by courtesy of the *Geological Magazine*.)

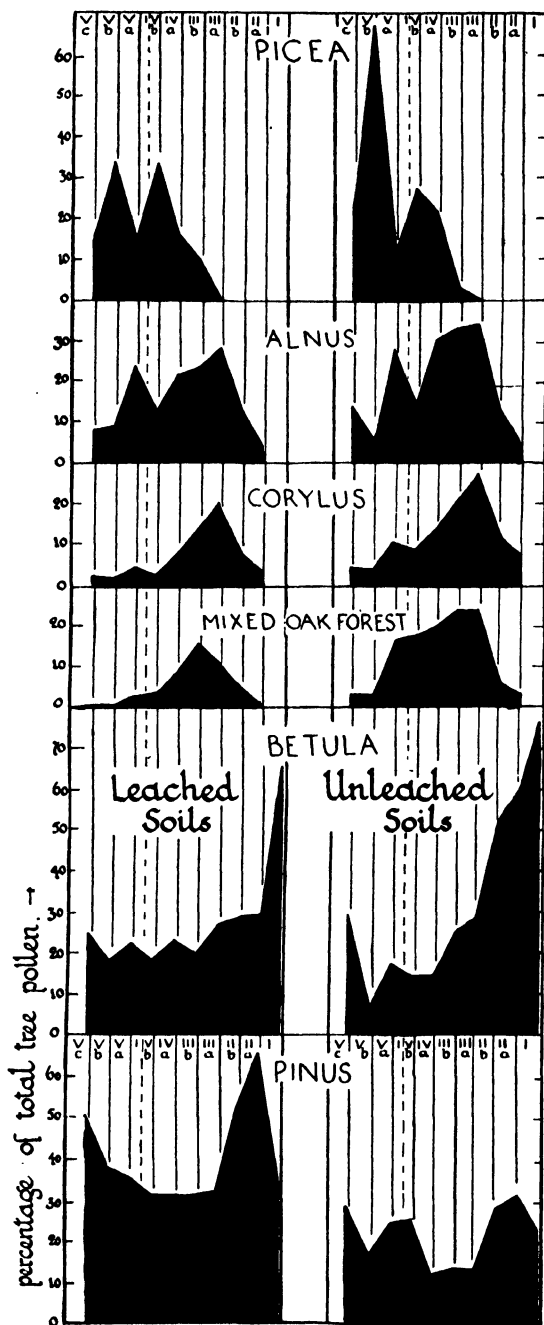
"carr" or scrub stage of this fen succession, along with *Alnus* and *Salix*, and there is evidence that *Pinus sylvestris* may in some cases enter the later phases. Thus the pollen of at least four tree species can be very greatly affected by local successional conditions, which may thus obscure very seriously the effects of the major climatic changes which form the basis for general correlation of the pollen analyses of an area.

One of the earlier American pollen-analysis papers (Lewis and Cocke (57)) gave results which were interpreted by the authors entirely on a successional basis, showing the transition of a swamp from open marsh conditions to a closed forest dominated by *Nyssa*, *Pinus*, *Chamaecyparis* and other trees, with indications of two more or less serious interruptions of the general development.

(b) Soil conditions

In his discussion of the regional development of the woodlands of Esthonia, Thomson in 1929 (73) drew attention to the consistent differences recognisable in two areas of the country with very different soils. His composite pollen diagrams for the two areas are reproduced in Fig. 11, the one for the leached scree and moraine soils within the lines of the Baltic transgression, and the other for the deeper unleached morainic soils lying farther inland. It will be seen that the pollen diagrams, though recognisably parallel with one another, differ in the same sort of way from one another right through the post-Glacial period. Thus on the poorer coastal soils pine is dominant throughout, but inland it is subordinated to other trees, in turn to birch, to the mixed-oak forest component and alder, and finally to spruce. The mixed-oak forests, in correlation with this, always maintain higher values on the better soils, though this is most marked during the Atlantic and sub-Boreal periods (phases IIIa to IVb). In the sub-Atlantic period the dominance of spruce pollen is very marked in the area of the good soils, where it reaches values of 70 per cent.; on the poorer soils it does not exceed the percentages already found in the sub-Boreal.

Similar conclusions as to the effect of soil conditions on former forest distribution have more recently been published by Hesmer for the area round Berlin (53). He compares the present distribution of natural forests with that indicated by the pollen diagrams and shows that all through the post-Glacial period, as now, the pine is dominant on the poorer soils. In the past, forests of the mixed-oak forest components, *Tilia*, *Ulmus* and *Quercus*, or, at a later date,



of *Fagus*, were dominant on the better soils where beech woods are dominant at the present day. Hesmer supplements these observations with a comparison of the superficial layers of bogs at places within and without the present beech area, and shows that at the present day high values for beech in the pollen rain are closely limited to the beech area. Hesmer also uses the pollen-analysis method to indicate that the areas now showing most development of heath are those which in the past have yielded the most abundant ericaceous pollen in the peat analyses.

In a country such as Britain, where many tree species show well-marked soil preferences, we may also expect such preferences to have left their mark in the regional differences between pollen-analysis sequences.

One is extremely tempted to ascribe to the soil conditions of extremely widespread waterlogging, the extremely high values of alder pollen which characterise so many British pollen analyses from the end of the Boreal period onwards. This effect is no doubt also partly a climatic one, but operating here mostly through soil factors. At the present day it is extremely difficult to realise the extent to which artificial drainage must have changed the natural water relations of the soils of this country.

(c) *Elevation*

The influence of elevation upon the present distribution of forest types is so strongly marked that its effects must always be looked for in the pollen diagrams of the more mountainous parts of Europe. In a country of such varying altitude as Switzerland correlation of pollen diagrams from different sites is impossible without recognition of the altitudes of the various sites in relation to the altitudinal forest belts of the present and of the past. In his masterly analysis of the post-Glacial forest history of the Alps Keller (56) has shown how consistently the forest belts can be recognised in each successive climatic period, and he effectively contrasts their altitudinal and temporal sequences on the north and south sides of the Alps.

Fig. 11. A dissection of two average diagrams from Esthonia (after Thomson), showing the complete post-Glacial sequence in pollen composition on two distinct types of soils, leached and unleached. In each case the roman numerals represent the successive phases of the post-Arctic period, namely: I, pre-Boreal; II, Boreal; III, Atlantic; IV, sub-Boreal; V, sub-Atlantic. The broken line in the later part of the sub-Boreal is the "Grenz-horizont." The pine, spruce and mixed-oak forest pollens are very differently represented in the samples from the two types of soil.

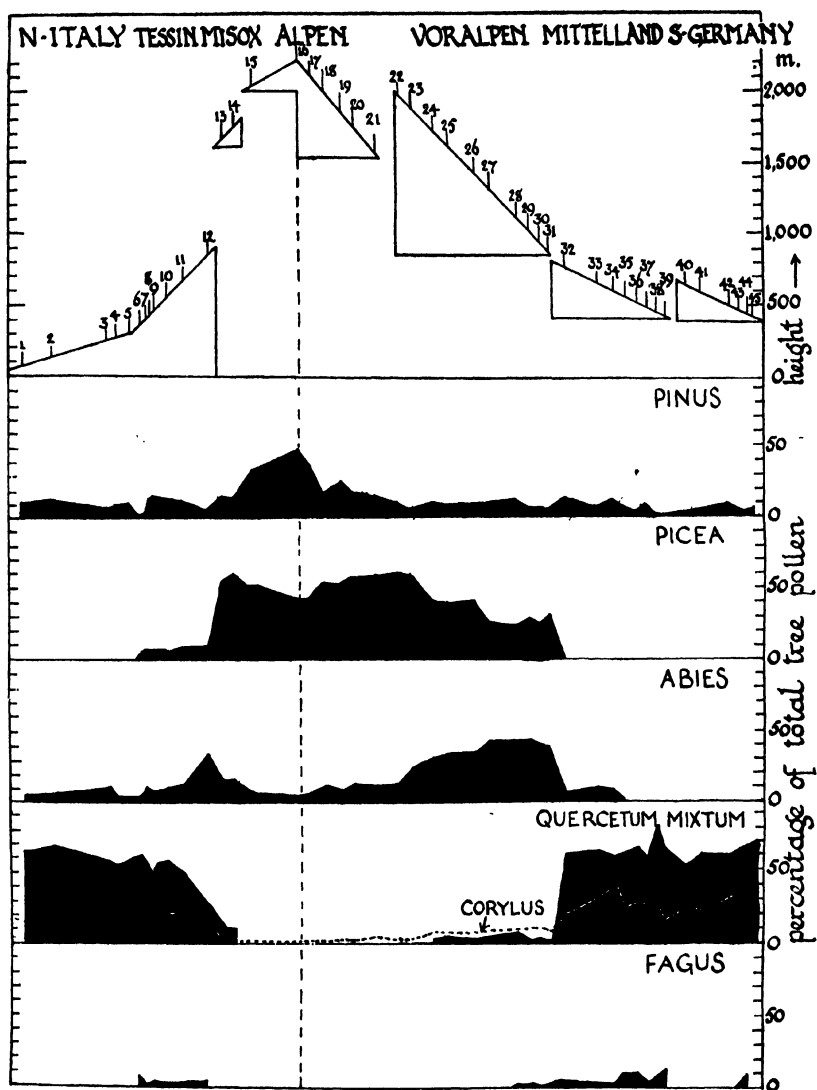


Fig. 12. In the upper part of the figure is a diagrammatic transect through the Alps to show the altitudes of 45 sites scattered across the Alps from south Germany to north Italy. At each site pollen analyses have been secured for the whole post-Glacial sequence and this figure deals with the results of comparing the pollen composition of all the sites at a given time—the time of the mixed-oak forest maximum. (Similar diagrams have been secured for the other phases of the post-Glacial period.) The lower part of the figure is a dissected pollen diagram showing the change of pollen rain at this time with varying altitude. Note especially that there is a pine maximum on the highest sites, below on each side of the Alps follow in turn maxima of spruce, fir, and mixed-oak forest with hazel. The beech is present at low altitudes but has not yet begun its phase of rapid extension and dominance. After Keller.

The diagram reproduced in Fig. 12 illustrates this relationship as reflected in the pollen analyses from the phase of dominant mixed-oak forests. The diagrams have been arranged by taking the results of analyses from forty-five sites distributed widely over the Alps from south Germany to north Italy. Though these sites by no means lie on a single bisect line across the Alps they have in the figure been grouped as if they do so, and in the upper part of the diagram the altitude of each site and its geographical position are schematically shown. The transect of the Alps so given is, of course, far from correct, but the relative slopes are fairly truthfully indicated. Though possibly somewhat displaced and almost certainly not indicating the proper absolute tree frequency, the pollen diagrams show a most convincing sequence of forest belts on each side of the Alps. The high Alps show a maximum of pine pollen (*Pinus montana* and *P. cembra*), and to north and south the spruce (*Picea*) reaches a markedly dominant position. Between about 800 and 1400 m. is indicated a belt of dominance of the fir (*Abies*), though this is particularly well marked in the Voralpen. Below 800 m. the forest composition shows a striking change from dominance by conifers to dominance by the deciduous components of the mixed-oak forest (*Quercus*, *Ulmus*, *Tilia*). The hazel (*Corylus*) pollen shows a similar curve, but also extends, probably in wind-carried grains, in small amounts right over the higher Alps.

Comparison of data such as this with similar data for the preceding and following forest periods affords the strongest possible type of evidence for former altitudinal movement of the forest belts. On these grounds Keller concludes that the forest belts were higher in the mixed-oak forest period than at any other time in the post-Glacial forest history of the Alps.

(d) *Regional parallelism*

Not only do the natural forests of Europe show an altitudinal zonation where sufficiently high mountains occur, but they show wide regional gradation. Particularly they show that the broad belt of temperate deciduous forest stretching from east to west across middle Europe gives place to the coniferous forest belt farther north. The distribution maps of the components of both the deciduous and coniferous forests show considerable internal variation in the present range of different tree species; and this variation appears to be an expression of response not merely to the general north to south temperature gradient across the continent but also to the east to west

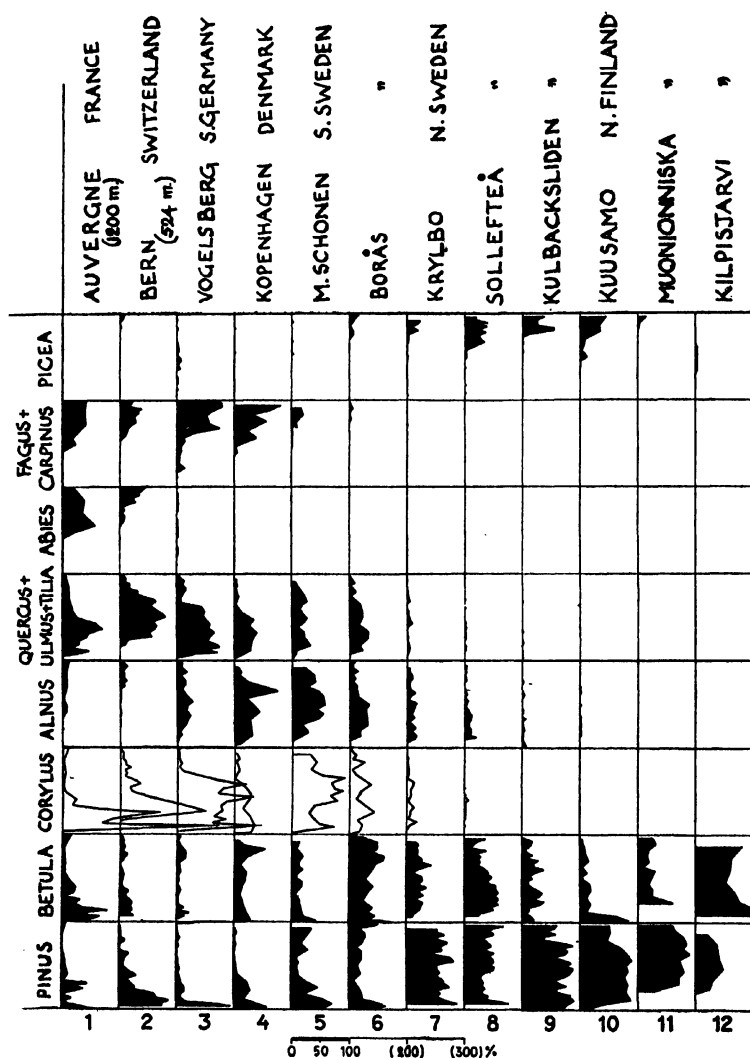


Fig. 13. A series of twelve pollen diagrams from sites along a north to south line from northern Finland to central France. Each rectangle includes the whole post-glacial sequence for one type of pollen. The effect of latitude in producing regional parallelism in forest development is extremely well shown. After von Post.

change from oceanicity to continentality of climate. In the same way that former altitudinal zonation is expressed in pollen diagrams of the post-Glacial period, so it has been shown are the wider regional distributions. These effects have been very beautifully demonstrated by von Post in a review of the present position of the interpretation of post-Glacial forest history of Europe from pollen-analytical data (62, 63, 64). Fig. 13 is a reproduction of one of his most striking figures. It is a longitudinal series of representative pollen diagrams from twelve sites along a line from central France to northern Finland: each diagram covers the whole period of post-Glacial forest history and together they give a means of examining the north-south gradation of European forests at each successive stage of the post-Glacial period. The middle stage shows very marked regional differentiation: in France and Switzerland the mixed-oak forests are overwhelmingly dominant, from middle Germany to southern Sweden the mixed-oak forests share dominance with the alder, in northern Sweden these warmth-demanding trees are almost absent and birch takes their place, while still farther north are striking pine maxima. This regional differentiation is conspicuous in the other phases of forest history also and involves other species than those just mentioned. Thus the hazel maxima which mark the beginning of the middle phase of the forest history are very strongly marked in the southern sites, whilst the pollen is nearly or quite absent from northern Sweden and northern Finland. The latest phase of the forest history is marked in central France and Switzerland by the rapid increase of the fir together with beech and hornbeam and in southern Germany and Denmark by the increase of beech and hornbeam alone. Farther north, in southern Sweden though beech and hornbeam increase there is also increase of pine, and in northern Sweden and part of northern Finland the increase is one of spruce and pine together. The most extreme northerly sites show an increase of birch.

An interesting complement of this longitudinal series is a similar composite diagram of a latitudinal series taken from west to east across Europe at the northern limit of the former zone of dominance of mixed-oak forests and alneta (Fig. 14). In contrast with the longitudinal series the component diagrams here show a very striking uniformity in general drift. In all save the most easterly there is recognisable the same entry, rise and fall of the mixed forest trees and of *Alnus*; von Post points out the special interest of the trees which especially characterise the periods immediately preceding the warm period and immediately following it. These species, *Pinus*, *Picea*,

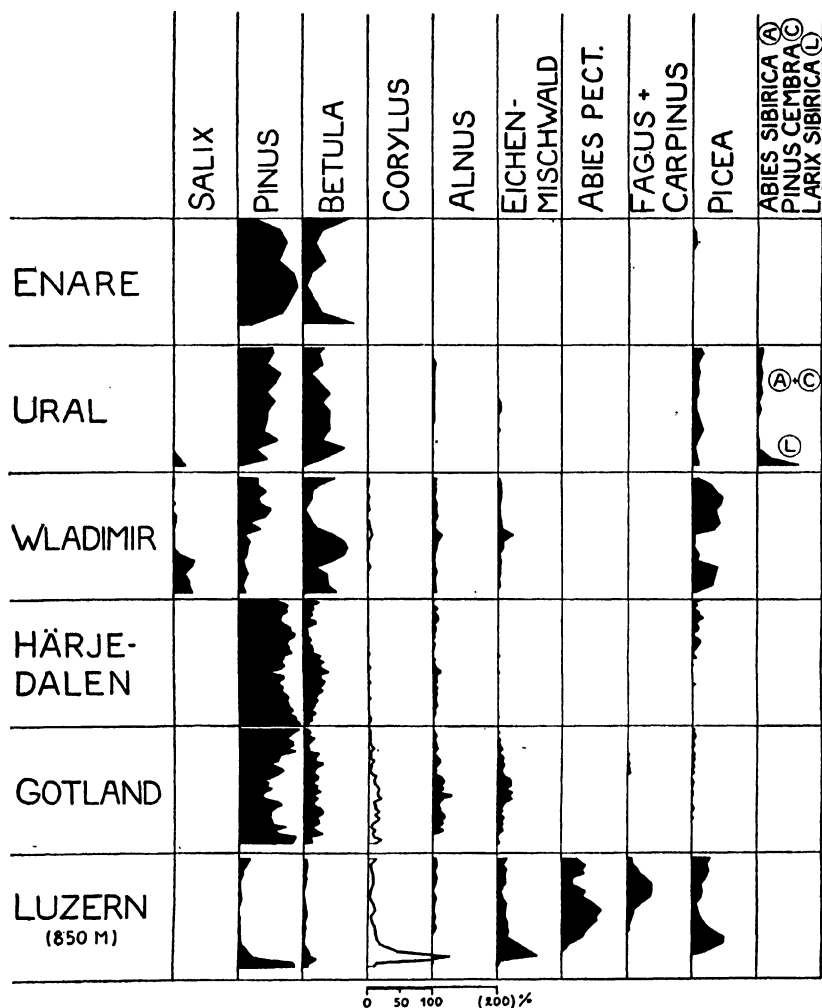


Fig. 14. A series of pollen diagrams from five sites along a line running from east to west across Europe rather north of the latitude of the middle of the mixed-oak forest zone. Luzern is a more southerly site included for comparison. The figures show striking uniformity in forest development, though the coniferous forest is progressively dominant in the east. All the sites show the "revertence" of coniferous forest during the last phase of the post-Glacial forest history. After von Post.

Abies sibirica, *Larix sibirica* and *Pinus cembra*, all show a marked retrogression during the warm phase and a subsequent movement towards recovery of their former dominance. This clearly implies that during the warm phase the coniferous forest retreated not only north but also east, and that in the succeeding period of diminishing warmth the elements of these forests have pressed in again to the south and west.

The diagram illustrates also other features such as the western limitation of the hazel, a point stressed by von Post and illustrated by cartograms showing the percentage frequency of hazel pollen in the post-Arctic period of increasing warmth.

The regional parallelism in development thus shown for the post-Arctic history of the forests of Europe can, whenever sufficiently intensive investigations have been made, be demonstrated also for much smaller regions. Thus von Post first demonstrated four types of parallel forest sequence in Sweden, the north Swedish, the west coast, the south Swedish hinterland and the Schona types. Among others Keller has demonstrated similar parallelism in Switzerland (55), Overbeck and Schmitz in north-west Germany (60), and Hesmer in the neighbourhood of Berlin. In view of the wide climatic differences across the British Isles we may expect clarification of our forest history to come through the elucidation of a similar parallelism in the different regions of this country.

It is perhaps hardly necessary to stress the fact that in the consistency and extent of this regional parallelism of forest development lies the fundamental confirmation and value of the pollen analytical method.

CORRELATION OF FOREST HISTORY WITH OTHER TIME INDICES

In so far as pollen analysis is a study of the temporal aspect of forest history its development is closely linked with all the sciences which concern also changes with time during the inter-Glacial or post-Glacial periods. Such sciences are especially those of geology, archaeology and climatology, and only by close and frequently revised correlation with the findings of these subjects can pollen analysis develop on the one hand as a means of tracing out in accurate detail vegetational history, or on the other as an auxiliary dating method for application to the special problems of these and related sciences.

(a) Geological

It so happens that in Scandinavia, the home of the invention and first intensive application of pollen analysis, there are two geological phenomena of outstanding importance as indices to phases of post-Glacial time. The first of these consists of the deposits and beaches of the successive phases of development of the Baltic Sea. The first such phases were the ice-dammed Baltic lakes. Melting of the ice barrier allowed communication with the Atlantic and formed the Yoldia Sea, which was marine and full of floating ice: it was characterised by the abundance of an arctic mollusc, *Yoldia hyperborea*. A rapid rise in the level of central Fennoscandia cut off the Baltic from the North Sea except by overflow channels in central Sweden and produced the Ancylus Lake, named from the small fresh-water mollusc *Ancylus fluviatilis*. This phase was followed by extensive digression and transgression of the sea during the *Littorina* period (*Littorina littorea*); connection with the Atlantic was re-established and the Baltic became salter than either before or at the present day. Southern forms of shell such as *Tapes decussatus* also indicated higher temperatures than those of the present day. This period was followed by the cooler and less saline conditions of the Limnaea Sea. The present period was reached by some re-elevation of the land, and the arrival of *Mya arenaria*, which gives its name to the period. Peat and other deposits occurring at critical levels in relation to the deposits of these seas, for example, upon their beaches, covered by their marginal deposits, or laid down in abandoned overflow channels, have undergone systematic pollen analysis, so that the phases of development of the Baltic lake have been very closely tied to the phases of development of the forest history of the surrounding countries (von Post (61), Erdtman (40)).

The second geological phenomenon of outstanding importance is the deposition of the glacial varves or lake deposits described by de Geer (78). These deposits of laminated clays owe their structure to the seasonal melting of the glaciers, causing streams to be fuller in summer and to deposit therefore coarser sediment than during the winter. By co-ordination of separate sections and by a single complete section de Geer has thus been able to establish a geochronological series for the whole of post-Glacial time. This sequence has been applied to the dating both of the Baltic lake periods and of the forest periods, and the general conclusion will be found set out in the table by Gross (52) (Fig. 15). The validity of de Geer's geochrono-

GEO-CHRONOLOGY	GEOLOGY		ARCHAEOLOGY.	FORESTS	CLIMATE	
	Baltic lake periods after Munchie & Sauramo.	Other geological events.			according to Gross (3).	periods according to Bluff & Sander.
after de Geer Sandersten Sauramo.			periods in Fennoscandinavia after Montelius.	of S. Sander, based on pollen analysis & macro-pollenology after von Post.		
1,000	MYA		Historical time	Beech & Spruce	2 climate of today	RECENT (warmer & dry)
0	PRESENT BALTIC		(Early) IRON AGE		climatic subglacial	SUB-ATLANTIC (cool & very wet)
2,000	LUMINAEA	'sub-boreal' - sub-atlantic near contact	BRONZE AGE	Mixed oak forests	Climatic 'subnormal' (becoming colder & drier)	SUB-BOREAL (dry, warm & continental)
4,000	2nd regression 1st Littorina Transgression	narrowing of the Danish Sund	Stone cists Passage graves Dolmens young & old kitchen middens = Campignian		warm & moist, more extreme seasons than now	ATLANTIC (warmer & moist) oceanic
6,000	Regression of Littorina Transgression Ancylus Maximum (fresh)	cutting off of the Baltic	Mesolithic	Hazel scrub Pine Birch & Pine	Post-glacial Mesolithic maximum warm continental	BOREAL (warm, dry & continental)
8,000	YOLDIA (salt)		Mesolithic		beginning of the warm period cool swampy, severe winter	SUB-ARCTIC
10,000	BALTIC ICE-DAMMED LAKE	ice margin of the Fennoscandian end moraines	END OF PALEOLITHIC	(Dryas flora)	cold & continental	ARCTIC
years.			GLACIAL FINI GLACIAL COTTI GLACIAL			

Fig. 15. Tentative correlation schema showing correlation of geochronology, geology, archaeology and the forest and climatic periods within the south Baltic region. Slightly modified from Gross. Reproduced by permission from Steers—Scott Head Island.

logical scale is generally accepted, and it is of particular importance since the earliest index otherwise available comes from the rather dubious northwards extension of Egyptian chronology, which in any case is not practicable before about 2000 B.C. (39).

Outside Fennoscandia similar instances of correlation of forest history and geological events have progressed much less rapidly. One of these instances is the application of pollen analysis to dating the submergence which caused the formation of the North Sea. It has been long known to the fishermen that peat, or "moorlog," forms the floor of large parts of the North Sea, especially in the region of the Dogger Bank. The plant content of this peat has been described by Mr and Mrs Clement Reid (67) and by Whitehead and Goodchild (77), but systematic pollen analyses were apparently first published by Erdtman in 1924 (41). Others have followed, and the table given on p. 341 indicates the nature of the results obtained.

With the exception of samples 7 and 11 there is a striking general uniformity in pollen content, which reflects the birch-pine woods of the Boreal period, the warmth-loving trees having as yet hardly appeared. The rising oak and higher hazel values in sample 7 suggest a somewhat later date, still, however, within the Boreal period, as possibly also does sample 11.

Towards the present coasts of the North Sea dredged-up peat samples of younger age have been described. Thus Lagerheim and von Post record pollen of alder, birch, hazel, pine, oak, lime, elm and willow from material found off the Swedish west coast. Numerous analyses made by Erdtman (42) and by Overbeck (59, 60) from dredger samples in the Jade-Weser estuary have a composition suggesting the early spread of the mixed oak forests at the beginning of the Atlantic period. It should be noted that beech and hornbeam are quite lacking even in these latter samples. The general indication appears to be that most of the North Sea was submerged during the Boreal period, and that to judge from the absence of overlying clays the sinking must have been rapid. Of deposits on the margin of the North Sea basin it is not yet possible to speak with any certainty. The peat beds and "submerged forests" found so abundantly round the British coasts at or below mean sea-level clearly hold the keys to these problems so far as they concern the western margin of the North Sea. Dr Vermeer-Louman has described similar deposits in the Netherlands. Pollen analysis makes it clear (H. and M. E. Godwin (48)) that the British submerged forests must be referred to at least three periods in post-Glacial time, so that the story of relative movement

Pollen analyses of samples of moorlog from the North Sea

No.	Long.	Lat.	Depth		Author	<i>Betula Pinus Alnus Ulmus Tilia Quercus Corylus</i> (birch) (pine) (alder) (elm) (lime) (oak) (hazel)							
			Fathoms	Metres		52	46	—	—	—	—	—	20
1	55° 10' N.	4° 20' E.	22	—	Erdtmann	52	46	—	—	—	—	—	20
2	54° 50' N.	4° 25' E.	29	—	"	2.5	97.5	—	—	—	—	—	1
3	55° 32' N.	4° 27' E.	18	—	"	15	85	—	—	—	—	—	—
4	55° 32' N.	4° 27' E.	18	—	"	—	100	—	—	—	—	—	—
5	54° 50' N.	4° 35' E.	22	—	"	21	79	—	—	—	—	—	4
6	54° 46' N.	4° 0' E.	28	—	"	16	84	—	—	—	—	—	8
7	55° 0' N.	4° 38' E.	23	—	"	12	74	—	2	—	12	47	—
	53° 10' N.	2° E.	19	—	Erdtmann	43	57	—	—	—	—	39	80 top
					H. and M. E. Godwin	16	64	—	—	—	17	80 bottom	—
					"	13	85	—	—	—	1.4	81 bottom	—
8	53° 10' N.	2° E.	19	—	H. and M. E. Godwin	30	69	1	—	—	—	4	—
					"	75	23	—	—	—	—	7	—
9	56° 25' N.	5° 55' E.	21	39	Vermeer-Louman ⁽⁷²⁾	52	43	3	—	—	—	4.7	—
					"	49	45	2	—	—	—	2	—
					"	27	73	—	—	—	—	1	—
10	"	"	"	"	"	38	59	2	—	1	—	19	—
					"	8	92	—	—	—	—	5	—
					"	6	94	—	—	—	—	8	—
					"	4.3	95.7	—	—	—	—	9	—
11	Unknown				"	3	27	68	—	—	1	—	—

of land and sea is by no means a simple one. Other aspects of the same problem have already been approached by analyses of peat beds upon or below the Scotch and Irish 25-foot raised beach and below the carse clays of the Scottish lowlands. At the same time the problem as a whole is barely touched and is here referred to only as an illustration of the application of pollen analysis to geological enquiries.

It need hardly be said that in the complementary sense also the internal correlation of pollen sequences in any given region will need to utilise fully whatever lines of geological evidence present themselves.

(b) Climatic

The connection of forest history with climatic change is of fundamental importance and the validity of pollen analytic methods depends largely upon the demonstration that changes in forest composition have been primarily determined by climatic causes. It has been often suggested that the rates of spread of trees, the distances from their glacial refuges and similar factors have been responsible for the major drifts of pollen-analysis curves, and that local factors such as soil, topography and elevation outweigh in effect the influence of general climatic change. These arguments, as we have shown in the previous section, are very strongly refuted by the phenomena of regional parallelism and of reversion. It has been very clearly demonstrated in Sweden especially that the spruce and beech expanded very rapidly and suddenly as forest-forming trees after a long period of sporadic occurrence in the country. Moreover, their advance was not continuous but intermittent. An examination of even these smaller movements leaves no doubt in the minds of Swedish workers that they are due to small climatic oscillations traceable throughout a large part of Sweden and perhaps beyond. Local topographic and edaphic influences are admitted to play a part in forest history but only in determining special local facies of the general climatic sequence.

Swedish forest history, even before the development of pollen analysis, had been developed in outline and applied to the post-Glacial climatic sequence proposed by Blytt and Sernander. With the extension of pollen analysis the correlation was much more closely and definitely determined, with the results shown in the table of Fig. 15. The Blytt and Sernander periods were as follows: (1) the pre-Boreal—sub-Arctic; (2) the Boreal—warm and dry; (3) the

Atlantic—still warmer and wet; (4) the sub-Boreal—warm and dry; (5) the sub-Atlantic—cold and moist. For southern Sweden the Boreal period was marked by the dominance of pine or pine and birch together, components of the mixed-oak forests and the alder were almost entirely lacking and the hazel was present in small amount. The transition to Atlantic time was marked by increase of the hazel to high values, by the slow increase of oak and elm and the rapid increase of alder at the expense of birch and pine. In middle Atlantic time came the culmination of the mixed-oak forests often with the lime particularly conspicuous. The sub-Boreal was marked by sporadic occurrence of spruce, beech and hornbeam. In the sub-Atlantic they all increased (beech especially in Schona), but beech and hornbeam retrogressed again with corresponding re-advance of the pine.

With greater or less success this climatic schema has been extended outwards from Sweden to other parts of Europe, and though often yielding a consistent picture of climatic and vegetational history it does not apply unmodified to the whole of Europe, nor is it adequate for the complexity of climatic history now recorded in Sweden itself. Von Post has, therefore, proposed a basic threefold division of post-Glacial time which is applicable to the whole of Europe and which corresponds to the main features of forest development:

“1. The stage of the approach of the warm period, characterised by the appearance and first increase of relatively heat-loving trees of different kinds.

“2. The stage of culmination of these forest elements.

“3. The stage of the decrease of the characteristic trees of the warm period and the appearance or the return of the dominant forest constituents of the present day.”

The use of such a schema for Europe as a whole has clear advantages over the Blytt-Sernander scheme, and it has already been adopted by some workers, notably Keller. It is perhaps an advantage that this scheme avoids one of the outstanding problems of post-Glacial climatology, namely the possibility of a secular dry period corresponding to the sub-Boreal of Blytt and Sernander. This problem is very closely bound up with the nature of the “Grenz-horizont,” which occurs with such frequency and regularity in the moors of western Europe that it takes front rank as a climatic horizon. It is usually represented by a line of junction between dark brown heavily humified *Sphagnum*

peat which is attributable to the Atlantic period and pale brown almost perfectly preserved *Sphagnum* peat of the sub-Atlantic period, though in many places layers of heath plants or stumps of trees are also held to show it. In the Blytt-Sernander scheme the *Grenz-horizont* was placed at the sub-Boreal sub-Atlantic contact, and it was assumed that it had been produced by the secondary destruction caused in the Atlantic *Sphagnum* peat by the warm dry climate of the sub-Boreal. The cold and moist climate of the sub-Atlantic was supposed to have brought about renewed formation of ombrogenous *Sphagnum* bog. Later workers have, however, placed other interpretations upon the *Grenz-horizont*, and despite its obviousness and wide occurrence no general agreement has yet been reached as to its climatic significance. Thus von Bülow(38) considers the strong destruction of the old peat not to be secondary but to be a primary character due to the bogs reaching the natural end of their developmental cycle, i.e. maximum convexity, during a warm dry period. Though also taking the view that the *Grenz-horizont* is primary, Granlund considers it due to a phase of arrest and erosion reached in a warm, wet, oceanic climate, and that renewed peat growth set in with lowered temperature, shortening of the vegetative period, lowered rainfall and increase in the period of frost. Gams(46) lays more stress upon the lowered rainfall than upon lowered temperature. It should also be recognised that as a result of very careful investigations of Granlund(51) there can be recognised in the bogs of southern Sweden no fewer than *five* horizons, all of the character of the *Grenz-horizont*, and several such horizons may occur in the same bog. Granlund regards each such "Rekurrenzfläche" as due to the bog having reached a phase of maturity and stagnation under given climatic conditions, and then having been started off again with renewed *Sphagnum* growth by a slight increase in precipitation. The five horizons he dates approximately as (1) c. A.D. 1200, (2) c. A.D. 400, (3) c. 600 B.C., (4) c. 1200 B.C., (5) c. 2300 B.C.

These brief comments will serve to illustrate the uncertainty which attaches to one of the most important climatic horizons of the bogs of western Europe. Much less doubt attaches to the general climatic drift, and especially to the high mean temperatures of the Atlantic climatic optimum. Evidence from all sides combines to show that the mean yearly temperature must have been some degrees higher than at present. Pollen analyses show not only the tree belts some hundreds of feet higher in the mountains of Europe than at the present day, but at the same time show the forest belts farther north

and east than at present; fresh-water plants and animals, marine organisms in the Baltic deposits, archaeological evidence of occupation of sites now much too far north or too high on mountain sides; all this and much more confirm the inference. The transportability of pollen makes caution advisable in drawing deductions from pollen series as to climate, but the cases of parallel forest development already mentioned seem to leave no alternative explanations.

It has been said that a complete knowledge of the plant covering of a region at any part of the post-Glacial period should allow, by recognition of the range of climatic tolerance of all the species, an exact reconstruction of the former climate. Though true enough in principle there are many difficulties in realisation of this end. Erdtman⁽⁴³⁾ has pointed out that each species population of the present day is a complex of ecotypes adapted to differing climatic and edaphic ranges, and arguments from one such ecotype cannot apply to the rest or to the aggregate species. The evolution of ecotypes is, however, continually progressing, and it is permissible to consider each species not to have exceeded in the past its present range of tolerance.

It is also extremely difficult to draw conclusions from the distribution diagram of a species from its autecology in any given region at the present day as to climatic causes for its former extension or retrogression. Thus the distribution and autecology of the beech is now, as a result of the co-ordinated work of the ecological section of the International Botanical Congress, probably better known than that of any European forest tree⁽⁶⁸⁾. It appears on the eastern limit of its distribution from Poland to the Black Sea to be controlled chiefly by summer dryness, winter frost and late spring frosts, its southern limit is set apparently by the dry and hot Mediterranean summer climate which causes the beech forests to be limited to very high mountain belts. On the north-west border in England and Sweden it is said that low summer temperatures prevent the formation of flower initials and proper ripening of mast, that late spring frosts injure the flower stigmas, and that in regions of high rainfall or poor soil, accumulation of leaves and soil degeneration sets in. It is clear that former retrogression of beech forests shown by pollen analysis could have very different climatic explanations along different parts of the periphery of the distribution area of the beech, and this tree which from its general European or British distribution might be called a "continental" type, might be called, by a central or east European botanist, an "Atlantic" type. Thus Bertsch⁽³⁷⁾, arguing from pollen analyses in south Germany, says that the climate

at the time of the maximum extension of the oak was warm, dry and continental, because the oak has a wide continental range...and that the extension of beech afterwards was due to increasing humidity, as the beech is a more Atlantic type. It is true that in the east the oak extends into much more continental climates than the beech, but in the west it also extends into much more maritime climates also. Such inferences from changes in vegetational cover need careful and wide confirmation and indeed require a wider knowledge of the autecology of tree species than we at present possess.

(c) *Archaeological*

As with geology and climatology, so also with archaeology pollen analysis may both assist and be assisted, according to which of the two subjects has already achieved for the given locality the closest chronological correlations. A particularly good example of the latter case is given by Bertsch's investigations in the deposits of the Federsee in Swabia (37). The Federsee is a small lake which since the Glacial period has been continually shrinking in area by the normal processes of marginal vegetational invasion and peat growth. Throughout this time prehistoric and historic man settled upon the lake margin and their occupation sites became embedded in the growing peat deposits. Extremely extensive excavations have revealed archaeological horizons of all periods from the end of the palaeolithic to the present day, and these have been described by Dr Reinherth. On the site of each discovery Bertsch made a vertical series of pollen analyses passing through the occupation horizon, and these series were supplemented by a series from borings designed to elucidate the structure and relations of the lake deposits. The extensiveness of the archaeological correlation can be judged from the fact that no less than fourteen Bronze Age sites were transected by pollen analysis series, and it is a striking proof of the validity of the pollen analysis method that in all these cases the pollen diagrams are not only extremely alike but are cut by the Bronze Age horizons at exactly equivalent levels. The final generalised diagram given by Bertsch for the forest history of the region is reproduced in Fig. 16. It will be seen that it represents the typical middle European sequence of forest horizons; the hazel maximum in the Tardenoisian, the beech maximum at the close of the Bronze Age, and the reversion of pine and spruce in historic time are very clearly shown.

An example of the complementary case of pollen analysis assisting archaeology is given by the dating of the celebrated Esthonian cul-

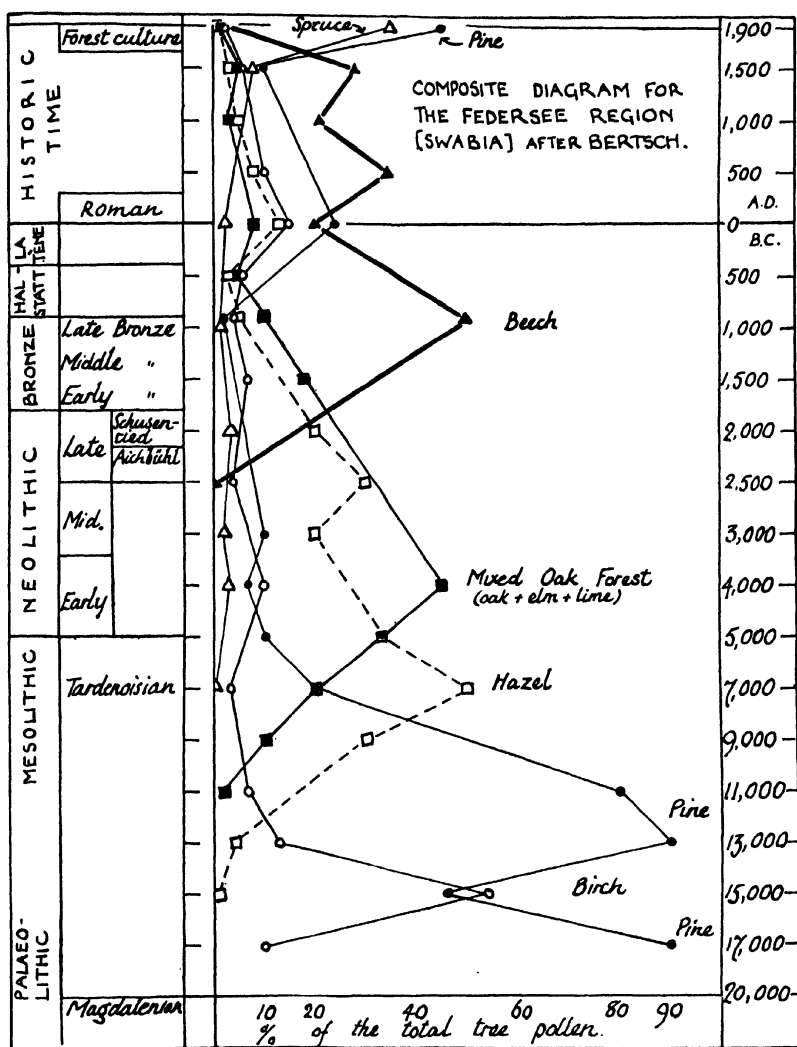


Fig. 16. Composite pollen diagram for the deposits of the Federsee region of Swabia, south Germany. It is based on analysis of a large number of vertical series of peat samples, and is very closely correlated with the very abundant horizons of human occupation which occur stratified in the lake deposits. After Bertsch, reproduced by permission from Steers—Scott Head Island.

tures at Kunda and Pernau by the analyses of Thomson (72, 74). The artefacts from Kunda came from a calcareous fresh-water deposit laid down in the shallow Kunda Sea above a clay containing a sub-Arctic flora, and overlaid by a sandy layer formed by the catastrophic invasion of the lake by the rising Baltic Sea. All these strata have been subject to careful pollen analysis with the results shown in Fig. 17. The artefact layer occurs at a readily recognisable horizon in the forest sequence of Esthonia, as shown in the left-hand diagram. It is the phase in which high pine-birch values are giving place to the slow increase in the newly immigrated alder, elm and hazel: it predates the entry of lime and oak. Thus a Boreal age can safely be attributed to it and this shows exact agreement with the Mesolithic Maglemose cultures from Müllerup, Svaerdborg and Holmegaard in Denmark, where the pollen analyses of the sites of the type stations were made by Jessen (54). By similar methods other cultures at Pernau and the Embachtal in Esthonia have been shown by Thomson to be of the same age, and it has recently proved possible to extend this dating to the sites of two harpoons of Kunda type recently found in the British Isles. The first of these was dredged up in moorlog found between the Leman and Ower banks off the Norfolk coast, and the results 7 and 8 given in the table on p. 341 are from two samples subsequently dredged from the same site and analysed by Erdtman and by H. and M. E. Godwin (49).

The other British site is at Skipsea on the Yorkshire coast, where marine erosion has exposed a section through the peat deposits of a former lake or mere. It is the basal silt of the mere which is alleged to have been the site of the discovery of a bone harpoon of Kunda type. Doubt has been cast on the authenticity of this discovery (69, 70, 71), but pollen analyses through the mere deposits indicate in a very striking manner that the basal silt horizon is certainly late Boreal in age as were the cultures to which this harpoon had been previously assigned. The analyses are shown in Fig. 7 (p. 293), and it will be seen that the forest characters of the late Boreal phase are all strikingly developed. The high *Corylus* percentages are particularly notable.

It is not our intention to quote in detail further cases to illustrate these applications of the pollen-analysis method to archaeology, but it is of some interest to note that already in two instances, the "Larne axes" on the 25-foot raised beach of the north of Ireland (44) and the industry at Lower Halstow (45), pollen analysis has been used to show the presence of Mesolithic cultures in the British Isles, at a period

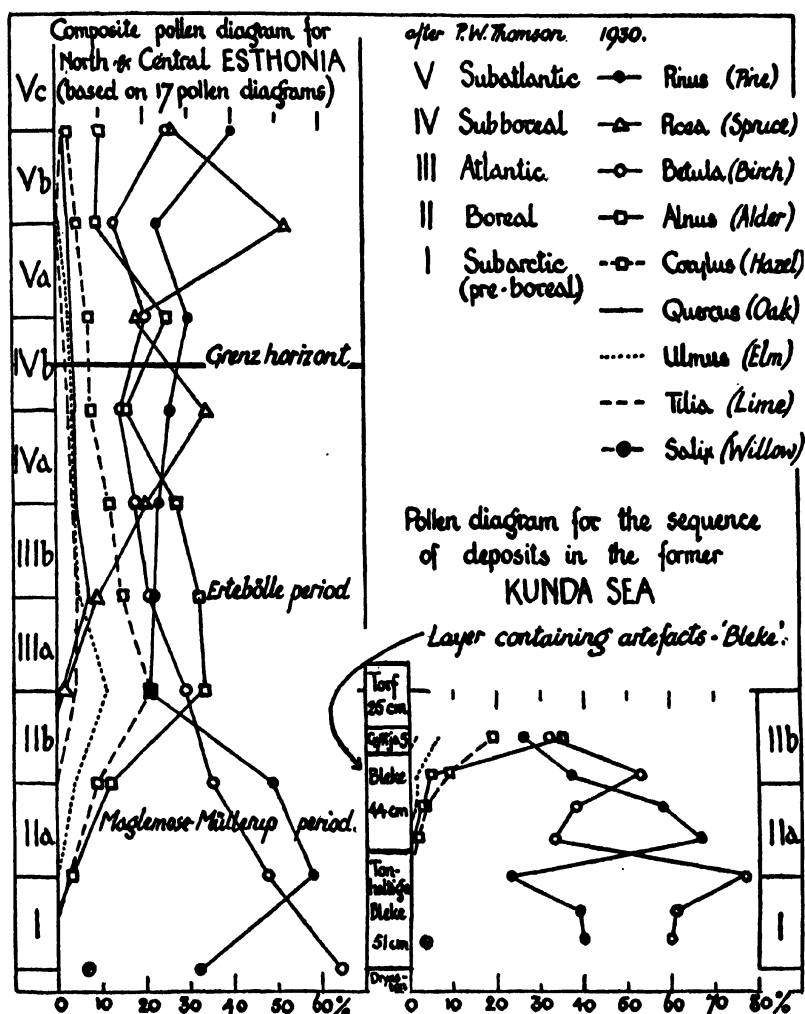


Fig. 17. On the left the generalised pollen diagram for the whole of north and central Estonia, correlated with the Blytt and Sernander climatic periods. On the right analyses through the artefact-containing layers of the former Kunda Sea. The Kunda artefacts are shown on this evidence to be of similar age to the Maglemose-Müllerup culture of Denmark. Reproduced by courtesy from *Antiquity*.

post-dating the Maglemose. In each case the trees of the warm period are present in peat beds older than the archaeological horizon, which must thus correspond to one of the later Mesolithic phases of Denmark and Scandinavia, such as that of the "kitchen-midden" culture, which is associated with the beaches of the *Littorina* maximum.

It may be pointed out that the usefulness of pollen analysis in archaeology may be much extended by the fact that very small samples are adequate for analysis. Thus the mud on the surface of a potsherd or the peat in the hollow of a bone may in some cases afford an adequate basis for dating a culture.

In those cases when pollen-analysis investigators look to archaeology for a chronological scale it should be noted that the archaeological horizons may in almost all cases be said to be sloping. The cultures of Europe spread out from Egypt and the East by the routes especially of the Danubian basin and the Mediterranean and Atlantic seaboard. Very often the diffusion of cultures was extremely slow, as is illustrated by the two following extracts from a paper by M. C. Burkitt and Professor Gordon Childe (39).

"It seems highly probable that both food-production and then organised industry and commerce began first in Egypt or Mesopotamia or in their vicinity and spread thence in very slow ripples so that Britain and Denmark might lag 2000 or more years behind." "The Iron Age begins then at different times as this secret spread out from the centre of discovery in Hittite Asia Minor during the 14th century B.C., reaching southern England, for instance, first towards the sixth century."

This implies that different cultures, i.e. the fashions of communities, coexist at a given time; thus, for instance, north-west Europe was still in the Stone Age when in Egypt the Bronze Age was drawing to a close, and iron was almost on the point of exploitation. This principle is also illustrated by the pollen-analysis data we have already given, which shows that during the hazel maximum at the end of the Boreal period there were Tardenoisian cultures in southern Germany and Maglemose cultures in Denmark, Sweden, Esthonia and parts of England.

It is possible to demonstrate that in all the sciences to which we have referred in discussing the correlation of the temporal sequences of post-Glacial time, in geology, climatology, archaeology and pollen analysis alike the same problem of sloping horizons recurs, though nowhere possibly so strongly as in archaeology. There is no simple

key to such a problem, but co-ordinated investigation should finally yield a simple system the internal coherence of which will be a major guarantee of its validity.

There are other fields of investigation than those already mentioned which also are concerned with the post-Glacial time sequences. These include the wide subjects of plant and animal geography, neither of which in the British Isles has yet been substantially co-ordinated with pollen analysis, though on the Continent much work has been initiated on these lines (see Bertsch⁽³⁷⁾, Gross⁽⁵²⁾, Gams⁽⁴⁶⁾), with results of considerable promise for future research.

APPLICATIONS OF POLLEN ANALYSIS TO THE BRITISH ISLES

The difficulties in applying to the British Isles the technique of pollen analysis are considerable. In the first place from our post-Glacial forest history *Picea* and *Abies* have been entirely absent, and these are trees which have been major indicators in the north-west European pollen diagrams in the post-Boreal period. The same is true to almost the same extent of *Fagus* and *Carpinus*. These trees now appear to form natural woodlands only within a restricted area in the south and south-east of Britain, and here, apparently since close to their climatic limit, show strong limitation to special types of soil, the beech especially, in a way they do not on the Continent. The employment of the times of immigration and spread of these trees as post-Boreal time horizons is therefore peculiarly limited, and to make the situation still more difficult, it is extremely doubtful when these trees could have spread westwards from the Continent, since land connection with the Continent, though extensive in Boreal times, must have largely disappeared during the Atlantic period. The final isolation of these islands is not yet dated and has not been related to the time of rapid expansion of the beech and hornbeam in the countries bordering the North Sea. Beech and hornbeam horizons in the British pollen diagrams must, therefore, receive independent correlation with other time indices before they can be safely linked to the Continental horizons for these trees. It should be noted (see Watt and Tansley⁽⁷⁶⁾) that it seems extremely difficult to confirm reports of *macroscopic* remains of the beech in archaeologically dated deposits, and the evidence of beech pollen in peat analyses from the English fenland⁽⁵⁰⁾ and elsewhere⁽⁶⁴⁾ is hardly yet strong enough to contradict finally the suggestion that the beech is a Roman or post-Roman introduction.

The absence or infrequency of the fir, spruce, beech and hornbeam in our post-Glacial forest sequence adds enormously to the difficulties of establishing direct parallels between forest development on the Continent and here. This difficulty is especially pronounced in the later phase of post-Glacial time. The early birch-pine phase, the immigration of the warmth-loving deciduous trees, the rapid spread of the hazel and the suppression of the pine by the extension of the deciduous oak, elm, lime and alder, all these are as clearly shown in this country as elsewhere, and on this account we can safely extend here the accepted forest horizons of the Continent and regard the parallelism in development as sufficient evidence of contemporaneity in establishing phases of pre-Boreal and Boreal age. Once, however, past the Boreal-Atlantic transition phase no new trees appear to serve as forest indices, and it becomes extremely difficult to parallel our forest development with that of the Continent. It becomes extremely dangerous therefore to extend, on a basis of comparison of pollen analyses alone, any post-Boreal horizon from a well-worked-out Continental area such as southern Sweden, directly to the British Isles.

Certain general considerations will serve to strengthen this contention. Thus the climatic changes which very largely determined the forest movements of Europe must have been shown in these islands in a form much modified by the influence of the Atlantic and the North Sea. It is likely that the climatic changes will be still recognisable, but not by the same indices as those evident in Continental countries, and by effects of different magnitude. It must also be recognised that across the British Islands from east to west there is an extremely wide climatic range, sufficient at the present day to determine the climax soil and vegetation type of the west to be oceanic *Hochmoor* even at sea-level, whilst that in the east is mixed-oak forest. Where forest indicators are few and climatic changes complex the danger becomes greater that successional changes initiated or controlled by either topographic causes (e.g. submergence and elevation in the Fenlands and Broads of East Anglia) or by climatic causes (e.g. erosion of upland peat and re-invasion by plants) may obscure the general drift of the forest development controlled by the general climatic shift.

It seems, therefore, particularly desirable that the post-Boreal story of development of the British forests should be directly linked to as many independent time indices as possible, whether geological, climatic or archaeological. The isolation of these islands from the

Continent tends, as we have already said, to make such indices also unreliable, but intensive correlation of pollen-analytic, geological, climatic and archaeological data should yield eventually some one connected story which applies equally well to all these aspects of post-Glacial time. The archaeological data will be particularly useful, since they increase in accuracy in the later post-Glacial phases, which are those of greatest weakness in the pollen-analytic schema.

As the independent time scale for post-Boreal British pollen analyses is established in this way it will be possible to see the European parallels and to use them. Though several writers, including the present author, have attempted to use direct parallelism with the Continent in dating British pollen diagrams of post-Boreal age, such can probably only usefully follow very intensive examination of the internal evidence for the dating of our peat deposits and the woodlands sequence of the country.

It is not our present intention to enter a detailed discussion as to the conclusions which can be drawn from the pollen-analysis work carried out up to the present in this country. At the same time, we have utilised some of this material for Figs. 18 and 19, which are included to demonstrate a well-known method of graphical presentation of pollen-analysis results over any geographical territory. The horizon of the intersection of the falling pine and rising alder pollen curves has been taken as the Boreal-Atlantic transition point and has been identified in the pollen diagrams of various workers in this country (Erdtman, Blackburn and Raistrick, Woodhead, H. and M. E. Godwin). Map 1 shows the pollen composition at each site shortly before this transition point, and Map 2 the composition shortly after. At each site the percentage of the pollen of each tree genus is shown by the size of a sector of the circle marked distinctively.

Although a general agreement in the alder and pine values is inevitable for each of the chosen periods, since the alder-pine relation has been made the criterion of the time division, nevertheless the maps show the prevalence of such high pine pollen percentages in the one case and of alder pollen percentages in the other, that these trees must have been widely dominant in the two respective periods. This certainly emphasises the importance of the Boreal-Atlantic transition. The uniformity which was guaranteed by the method to the alder and pine pollen percentages will be seen from the maps to extend also to the other forest trees and to the hazel. Thus the Boreal diagram shows five sites with hazel percentages of more than 100, and in the original pollen diagrams similar high hazel values occur also in other

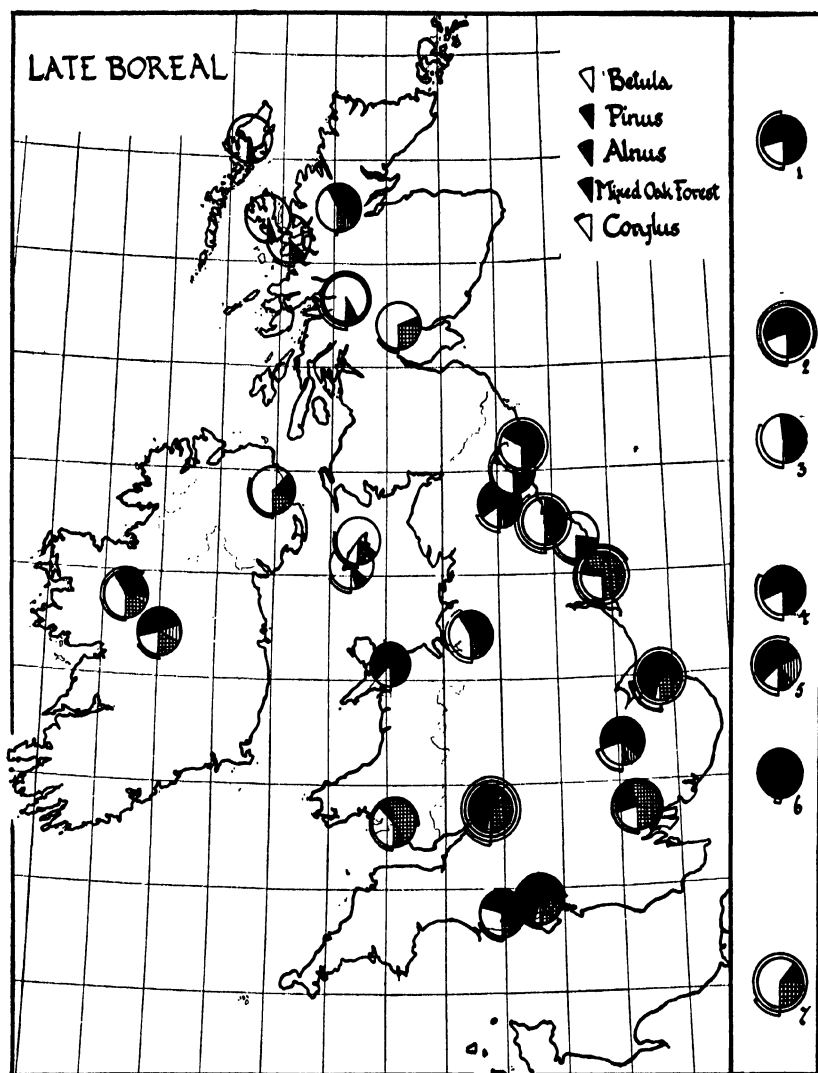


Fig. 18.

Figs. 18 and 19. Diagrams showing the percentage pollen composition of samples of late Boreal (Fig. 18) or early Atlantic (Fig. 19) age, from sites in the British Isles investigated by Erdtman, Raistrick and Blackburn, N. Woodhead, and H. and M. E. Godwin. The criterion of the Boreal-Atlantic transition has been taken as the intersection of the falling pine and rising alder pollen curves. The sites at Swansea (south Wales) and Cheltenham (Glos.) are exceptional in that the analysis shown is not part of a vertical series of analyses so that the exact validity of the dating is questionable. The percentages of the different types of pollen shown are indicated always in the same clockwise order, and the size of each sector is

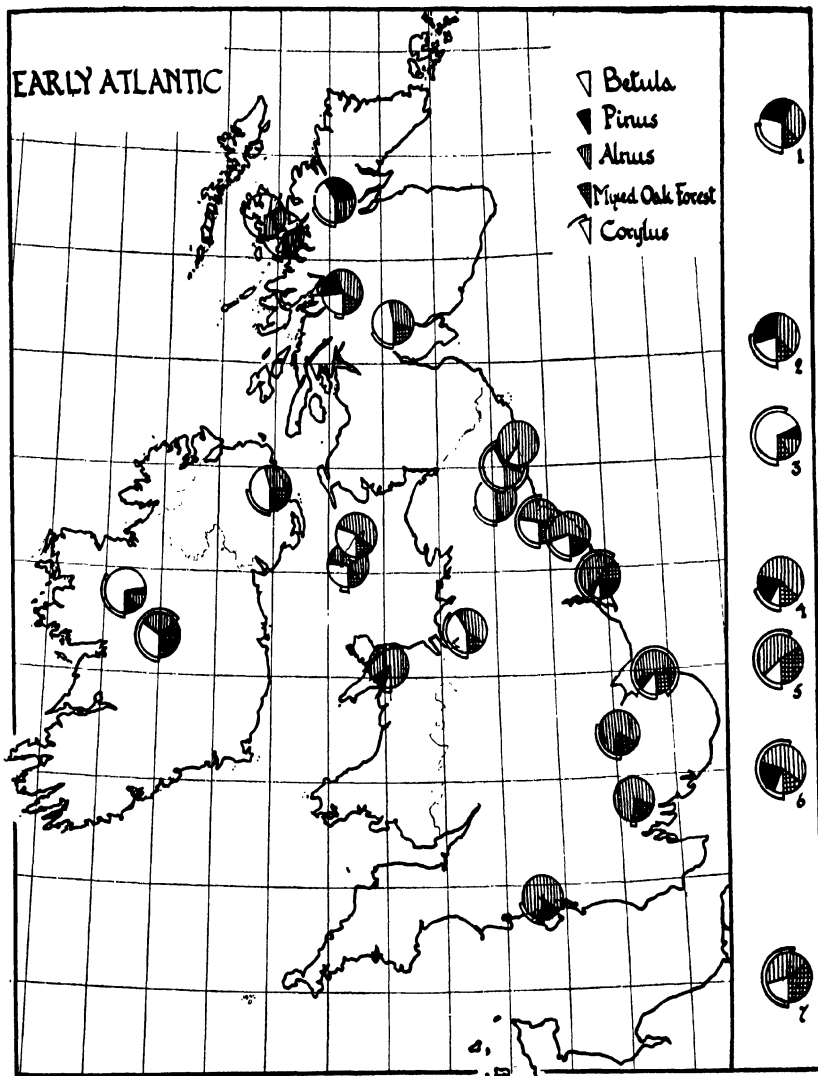


Fig. 19.

proportional to the percentage of each type of tree pollen present. Hazel pollen, which is reckoned separately, is expressed as a percentage of the total tree pollen. The columns on the right of each figure are analyses of Comparable age from lowland stations on the North Sea border of the Continent and each is shown approximately at its proper latitude. They are (1) Göteborg, Sweden; (2) Schona, south Sweden; (3) Zealand, Denmark; (4) Dannenberg, north-west Germany; (5) Valthermond, Holland; (6) Soesterveen, Holland; (7) south Belgium. After von Post, Jessen, Overbeck, Vermeer-Louven and Erdtman.

sites at horizons very near to this. On the other hand, in the early Atlantic map there is no instance of 100 per cent. hazel pollen, and in most cases there is a substantial decrease from the former values for the same site.

Another feature of extreme interest shown by the maps is a general drift of the pollen composition from north to south in these islands, coupled with regional parallelism of development. The Boreal map shows a very strong preponderance of birch pollen in north Scotland and especially the western isles, whereas the south is characterised by higher values for mixed-oak forest pollen. In the early Atlantic map it will be seen that birch pollen is absent or scarce in the southern sites whilst increasingly abundant to the north; pine in substantial amount is limited to Scotland.

The symbols set out in the right-hand column of each map are corresponding analyses from lowland sites on the continental margin and each is set approximately at its proper latitude. They range from south Belgium to Göteborg in Sweden (see legend to figures), and it is remarkable how definitely they are in general agreement with the British results. Note especially in the Boreal diagram the high *Corylus* values and the higher mixed-oak forest component in the southern sites, and the higher pine and birch values in the northern sites of the early Atlantic map.

These maps are of course at present very tentative, they show much too large lacunae, but even so show well enough one method of presenting regional data and the coherence of such a presentation. Continued work may be expected to yield similar and fuller maps of forest distribution at all stages of post-Glacial history for comparison and correlation with the already advanced series of such maps now available for most of the European continent.

In conclusion the author desires to thank Mr N. Woodhead for allowing the use of unpublished data on the north Wales peats for Figs. 18 and 19, and to thank the authors and editors who have kindly allowed reproductions of their diagrams to be made.

REFERENCES

- (37) BERTSCH, K. Paläobotanische Monographie des Federseerieds. *Bibl. bot.* 103. 1931.
- (38) VON BÜLOW, K. Allgemeine Moorgeologie. *Handb. der Moorkunde*, 1. Berlin, 1929.
- (39) BURKITT, M. C. and CHILDE, V. G. A chronological table of pre-history. *Antiquity*, 6. 1932.

- (40) ERDTMAN, G. Tapesgränser på Jaederen och dess relation till skogarnas historia i sydvästra Norge. *Svensk bot. Tidskr.* **21.** 1927.
- (41) ——— Some microanalyses of "moorlog" from the Dogger Bank. *Essex Nat.* **21.** 1925.
- (42) ——— En pollenanalytisk undersökning av torvprov från Jadenbukten och Weserestuariet. *Svensk bot. Tidskr.* **21.** 1927.
- (43) ——— Some indications of the character of climate and vegetation in north-western Europe during the Mesolithic Age. *International Congress of Pre-historic Archaeology.* London, 1932.
- (44) ——— Studies in the post-Arctic history of the forests of north-western Europe. I. Investigations in the British Isles. *Geol. Fören. Stockh. Förh.* **50.** 1928.
- (45) ——— in BURCHELL, J. P. T. A final account of the investigations carried out at Lower Halstow, Kent. *Proc. Prehist. Soc. East Anglia,* **5.** 1928.
- (46) GAMS, H. Neue Beiträge zur Geschichte der Ostsee. *Int. Ref. Hydrobiol.* **26.** 1931.
- (47) GODWIN, H. and M. E. Pollen analyses of fenland peats at St Germans, near King's Lynn. *Geol. Mag.* **70.** 1933.
- (48) ——— *Pollen analysis of peats at Scott Head Island, Norfolk, in "Scott Island,"* Norwich. 1934.
- (49) ——— British Maglemose harpoon sites. *Antiquity,* **7.** 1933.
- (50) ——— ——— ——— Unpublished data.
- (51) GRANLUND, E. De Svenska Högmossarnas Geologi. *Sverig. geol. Unders. Afh.* No. 373. 1932.
- (52) GROSS, H. Das Problem der nacheiszeitlichen Klima und Florenentwicklung in Nord- und Mitteleuropa. *Beih. z. Bot. Centralbl.* **47.** 1931.
- (53) HESMER, H. Die natürliche Bestockung und die Waldentwicklung auf verschiedenartigen märkischen Standorten. *Z. Forst- u. Jagdw.* 10-12. 1933.
- (54) JESSEN, K. Conditions géologiques des deux stations de plus ancien âge de la pierre dans la tourbière de Holmegaard. Extrait des *Mém. Soc. Roy. des Antiq. au Nord.* 1926-7.
- (55) KELLER, P. Die postglaciale Entwicklungsgeschichte der Wälder Mitteleuropas. *Rep. Proc. 5th Int. Bot. Congress, Cambridge.* 1930.
- (56) ——— Die postglaciale Entwicklungsgeschichte der Wälder von Norditalien. *Veröff. geobot. Inst. Rübel,* **9.** 1931.
- (57) LEWIS, I. F. and COCKE, E. F. Pollen analysis of Dismal Swamp peat. *J. Elisha Mitchell sci. Soc.* **45.** 1929.
- (58) MACFADYEN, W. A. The Foraminifera of the fenland clays at St Germans, near King's Lynn. *Geol. Mag.* **70.** 1933.
- (59) OVERBECK, F. Bisherige Ergebnisse der botanischen Moorforschung zur Frage der Küstensenkung an der deutschen Nordsee. *Abh. naturw. Ver. Bremen,* **29.** 1934.
- (60) ——— and SCHMITZ, H. Zur Geschichte der Moore, Marschen und Wälder Nordwestdeutschlands. I. Das Gebiet von der Niederweser bis zur unteren Ems. *Mitt. d. Provinzialstelle f. Naturdenkmalpflege, Hannover,* **3.** 1931.
- (61) VON POST, L. Svea älvs geologiska tidsställning. En pollen-analytisk studie i ancylustidens geografi. *Sverig. geol. Unders. Afh. Ser. C,* 347. 1928 (English summary).
- (62) ——— Die postarktische Geschichte der Europäischen Wälder nach der vorliegenden pollendiagrammen. *Medd. Stockh. Högsk. geol. Inst.* **16.** 1929.
- (63) ——— Die Zeichenschrift der Pollenstatistik. *Geol. Fören. Stockh. Förh.* **51.** 1930.
- (64) ——— Problems and working lines in the post-Arctic forest history of Europe. *Rep. Proc. 5th Int. Bot. Congress, Cambridge.* 1930.

- (65) RAISTRICK, A. and BLACKBURN, K. B. Pollen analysis of the peat on Heathery Burn Moor, Northumberland. *Proc. Univ. Durham phil. Soc.* **8**. 1931.
- (66) ——— The late-glacial and post-glacial periods in the North Pennines. Pt III. The post-glacial peats. *Trans. North. Nat. Un.* **1**. 1932.
- (67) REID, C. *Submerged Forests*. Cambridge, 1913.
- (68) RÜBEL, E. Die Buchenwälder Europas. *Veröff. geobot. Inst. Rübel*, **8**. 1932.
- (69) SHEPPARD, T. The Maglemose harpoons. *The Naturalist*. 1923.
- (70) ——— Yorkshire peat beds. *The Naturalist*. 1930.
- (71) ——— Maglemose harpoons. *The Naturalist*. 1930.
- (72) THOMSON, P. W. Das geologische Alter der Kunda- und Pernaufunde. *Beitr. Est-, Liv- u. Kurb.* **14**. 1928.
- (73) ——— Die Regionale Entwicklungsgeschichte der Wälder Estlands. *Acta et Comm. Univ. Tartuensis*, A, **17**, 2. 1929.
- (74) ——— Geologische Datierung archäologischer Funde in Estland. *Fornvännen*. 1930.
- (75) VERMEER-LOUMAN, G. G. *Pollen-analytisch Onderzoek van den west-nederlandschen Boden*. Amsterdam, 1934.
- (76) WATT, A. S. and TANSLEY, A. G. British beech woods. *Veröff. geobot. Inst. Rübel*, **8**. 1932.
- (77) WHITEHEAD, H. and GOODCHILD, H. H. Some notes on moorlog. *Essex Nat.* **16**. 1909.
- (78) WRIGHT, W. B. *The Quarternary Ice Age*. London, 1914.

Sources of further references

- ERDTMAN, G. Literature on Pollen-Statistics published before 1927. *Geol. Fören. Stockh. Förh.* 1927.
- Literature on Pollen-Statistics published during the years 1927–9. *Geol. Fören. Stockh. Förh.* 1930.
- Literature on Pollen-Statistics and related topics published 1930 and 1931. *Geol. Fören. Stockh. Förh.* 1932.
- Literature on Pollen-Statistics and related topics published 1932 and 1933. *Geol. Fören. Stockh. Förh.* 1934.
- GAMS, H. Nachträge zum Verzeichnis der pollenanalytischen Literatur. *Z. Gletscherk.* **15**. 1927.
- Zweiter Nachtrag zum Verzeichnis der pollenanalytischen Literatur. *Z. Gletscherk.* **17**. 1929.
- Dritte Nachtrag zum Verzeichnis der pollenanalytischen Literatur. *Z. Gletscherk.* **19**. 1931.

THE MORPHOLOGY OF THE LEMMA IN GRASSES

By W. R. PHILIPSON

The Royal Botanic Gardens, Kew

(With 7 figures in the text)

INTRODUCTION

IT has long been established that the lemma or flowering glume in grasses represents a bract, homologous with a vegetative leaf but specialised for flower protection. There never had been much doubt of this, although some morphologists thought that it might, with the palea, constitute a perianth, until Turpin (1819) showed that the lemma is inserted upon the rhachilla and not upon the floral axis. On the other hand, there has been some difference of opinion as to the parts taken in the lemma by the various regions of the typical grass leaf, viz. sheath, blade, and ligule. The most generally accepted view is based on the general resemblance between a foliage leaf and a lemma with a dorsal awn; the awn is homologised with the blade, and the parts of the lemma above and below the insertion of the awn with the ligule and sheath respectively. When the awn is terminal the whole of the lemma is considered as the sheath, and its lateral lobes, if any, as stipules which by fusion are supposed to give rise to the ligule. This interpretation, which has become the classical one, first propounded clearly by van Tieghem in 1872, was based on the anatomical work of Duval-Jouve (1871) and was later supported by Celakovsky (1897) where figures explaining this view will be found (Taf. IV, figs. 25-8). Hackel in *Die Natürlichen Pflanzenfamilien* accepted this theory and has been followed by the majority of botanists.

In 1921 Bugnon attacked this interpretation. He contrasted the ligule of the vegetative leaves with the upper parts of the lemma in awned grasses, pointing out that in *Avena* the ligule is always nerveless but the lemma strongly nerved, and conversely that in *Oryza* and *Ammophila* the ligule is large and strongly nerved but the corresponding part of the lemma is very reduced or absent. He contends that the nervation of the ligule is always by accessory nerves laid down late in the development of the leaf, whereas the nerves of the

lemma are principal nerves. Further he states that the lemma never has a closed sheath even in grasses such as *Melica* where the vegetative sheath is constantly closed. Finally in the series of leaves from the prophyll towards the inflorescence, the sheath, at first constituting practically the whole leaf, becomes more and more subordinate to the blade. From this evidence Bugnon concludes that the ligule is not homologous with the fused lateral lobes of the lemma, and also that the whole of the lemma is homologous with the blade of the vegetative leaf, the sheath being quite absent.

While supporting Bugnon in his attack on the classical interpretation of the morphology of the lemma, I cannot accept his conclusions. His interpretation of the lemma rests on the view that the ligule receives only accessory nerves. Some preliminary work on the development of the vascular strands in the ligule of *Deschampsia caespitosa* indicates that the nerves which enter the marginal part of the ligule appear in the normal sequence of the formation of the foliar vascular arc and not later than would be expected of marginal nerves as is implied by Bugnon. If this is the case there is no *a priori* reason to deny that the lateral lobes of the lemma might be homologous with the lateral region of the ligule in those grasses in which the ligule is nerved.

In the hope of settling this question I examined spikelets of several species of grass which show proliferation (so-called vivipary). The study of the transitional structures between the lemma and the foliage leaf leaves little doubt on the following points:

- (1) The dorsal awn is not homologous with the whole of the blade.
- (2) The sheath is represented to some degree in the lemma.
- (3) The marginal part of the ligule is in some cases represented by the lateral lobes of the lemma.

THE STRUCTURE OF PROLIFERATING SPIKELETS

General accounts of proliferation in grasses are given in the standard works on plant abnormalities (Masters, 1869; Frank, 1880; Penzig, 1922; Vuillemin, 1926). Frank distinguishes between spikelets in which the flowers, as he considered, become transformed into bulbils which may separate to form new plants, and spikelets in which the rhachilla is greatly prolonged. Penzig gives a systematic account with many references to authors who report proliferation in each species. Eichler (1881) and Schuster (1910) also give short reviews of the subject. Recently more intensive studies have been carried out dealing with single species. Exo (1916) gives an account

of the bulbils of *Poa alpina*, Evans (1927) describes proliferated spikelets of *Phleum pratense* under three heads: (1) those with a single enlarged lemma; (2) those produced as leafy shoots; (3) those with an elongated rhachilla. This last type was recorded and figured by Toumey in 1891; it has an internode of the rhachilla extremely elongated bearing distally either a normal lemma and flower or a leaf-like structure. The first two types of *Phleum* described by Evans are claimed by him to be "fundamentally different," since in the first type there is a single enlarged lemma with floral structures in its axil, and in the second the leaves of the proliferated shoot have no trace of floral organs in their axils; because of this Evans evidently supposes the leafy shoot of the second type to be due to a growing up of the floral axis rather than of the rhachilla. Arber (1928) and Thoenes (1929) give accounts of proliferation in *Cynosurus cristatus*, and here, as might be expected since the bulbils occur only at the extremities of the sterile spikelets, there are no floral structures in the axils of the leaves. Jenkin (1921) and Turesson (1926, 1930) have both published accounts of "vivipary" in *Festuca ovina*, but are concerned more with the ecological and genetical aspects of proliferation than the morphological. Both, however, stress the wide range of types to be found in this species.

Proliferation then can produce very varying effects on the structure of grass spikelets; some authors would consider these variations as due to fundamentally different causes. It is probable that the main axis of all such spikelets is a continuation of the rhachilla, and all leaf-like structures borne upon it are equivalent to lemmas. In the axils of these lemmas, flower rudiments are sometimes present or these may be replaced by lateral branches. If the internodes of the prolonged rhachilla are short and all or most of the lemmas leafy a bulbil is formed, and it may be taken as a general rule the larger the leaf the smaller is the flower in its axil, but even well-developed bulbils may contain floral organs in some species, e.g. *Deschampsia caespitosa* and *Poa alpina*. On the other hand when the rhachilla is prolonged with long internodes there is a greater tendency for flower production; in such cases the rhachilla may be leafy below and bear flowers or even spikelets above, or may be quite devoid of any leaf-like structures at all. Several of these types may be found associated together in the same inflorescence (Fig. 1 and Evans, 1927, Pl. 10c) and seem to be different effects of the same general cause. The bulbil is of some ecological importance and in some species it is part of the hereditary constitution of the plant (Jenkin, 1921; Turesson, 1926, 1930).

Morphological argument based on abnormalities are frequently of very doubtful value. This does not seem to be the case in the present instance, for proliferation in grass spikelets involves no very radical metamorphosis. It seems best to consider the lemma rudiment as multi-potential (Forster, 1928), developing normally into a lemma but under certain conditions into a foliage leaf. It is therefore inevitable that the regions of the foliage leaf are present potentially in the lemma rudiment and may develop into regions of the mature lemma. A study of the intermediate structures will indicate the homologies of these regions of the lemma and of the foliage leaf.



Fig. 1. *Deschampsia caespitosa* Beauv. Types of proliferating spikelets from the same inflorescence. 1 and 2, $\times 12$; 3, $\times 8$.

Such figures as have been published in the above-mentioned and other more purely taxonomic works in most cases show only the external aspect of the spikelet. Jenkin (1921) gives several diagrams of the disposition of the rachilla of the various glumes, but the only authors to figure the individual lemmas, showing their transition to leaves, are von Mohl (1845) and Stebler and Schröter (1889), both in the case of *Poa alpina*. From his investigations on this species von Mohl concludes that the sheath and the blade are both represented in the lemma, and the membranaceous border of the lemma stops short of the blade, its upper border forming the auricles. In view of this general neglect and for comparison with von Mohl's results,

descriptions and figures are given of the structure of a range of spikelets from two species, viz. *Dactylis glomerata* and *Deschampsia caespitosa*. Dissections were made of several other species, but they showed no essential differences from those described, which are sufficient for the present purpose, i.e. the elucidation of the morphology of the lemma.

Dactylis glomerata L.

This grass was investigated because so much of Bugnon's work was carried out on this species. Proliferated spikelets are frequently found in the autumn, they are usually only slightly modified as in

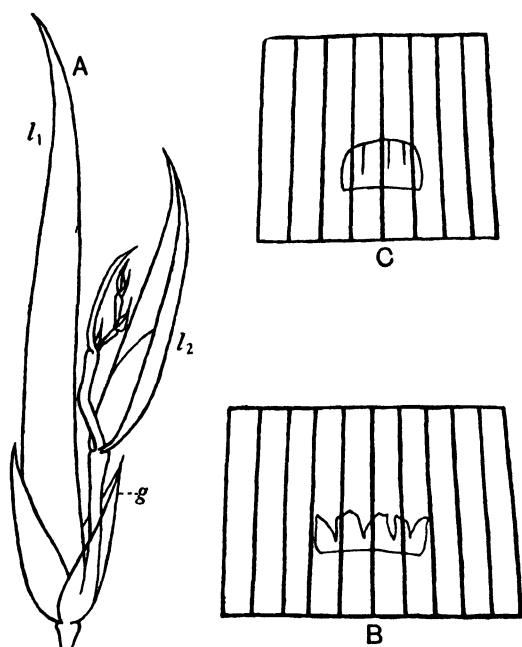


Fig. 2. *Dactylis glomerata* L. Proliferating spikelet 1. A, the spikelet, $\times 10$; g, glumes; l_1 , l_2 , first and second lemmas. B and C, membranaceous scales on ventral surfaces of l_1 and l_2 respectively; $\times 30$.

the first spikelet to be described, but, as in the second, occasionally they may show very striking development.

Spikelet 1 (Fig. 2A). The sterile glumes (g) remain practically as in a normal spikelet, except that they may be very slightly enlarged. The lowest lemma (l_1) is excessively developed, being about four times its normal length, and having nine instead of the usual five nerves. On the shoot in its axil is a two-nerved palea rather larger than normal,

enclosing a perfect flower. At the next node of the elongated rhachilla is a similar but smaller lemma (l_2) with seven nerves, which also encloses a palea and a complete flower in its axil. The rhachilla is prolonged and bears distally a cluster of four imperfect flowers, each with a lemma and palea, much reduced, and completely aborted sexual organs. In other spikelets from the same inflorescence in which the rhachilla was slightly more elongated this terminal group consisted of minute spikelets, for each was provided with two sterile glumes.

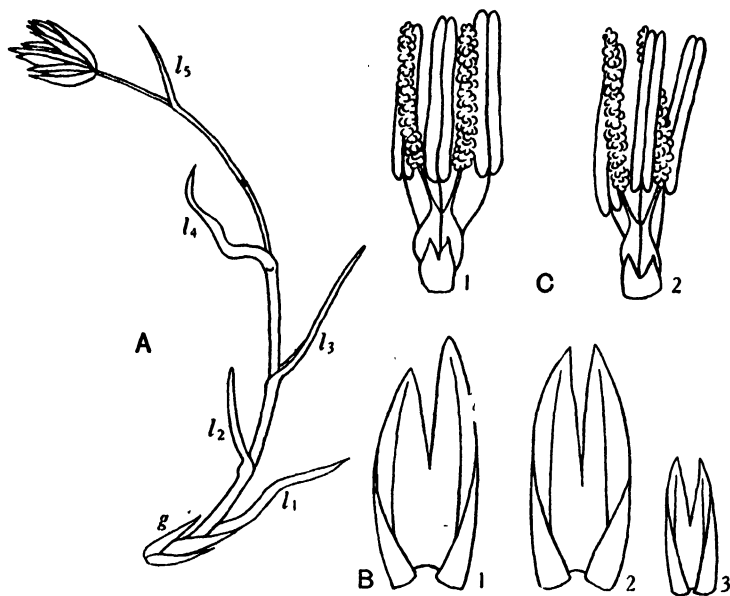


Fig. 3. *Dactylis glomerata* L. Proliferating spikelet 2. A, the spikelet, $\times 2$; g, glumes; l_1 - l_5 , 1st-5th lemmas. B, paleas enclosed by l_1 - l_3 , $\times 20$. C, floral organs in axils of l_1 , l_2 , $\times 20$.

Upon the ventral surface of the two enlarged lemmas, about two-thirds of their length from the base, were minute membranaceous scales (Fig. 2B, C) more or less torn inserted medianly.

Spikelet 2 (Fig. 3A). Again the sterile glumes (g) remain unaltered. The first lemma (l_1) closely resembles that in the spikelet previously described, but is larger. On the shoot in its axil is a palea (B 1) and a complete flower (C 1) and on its ventral surface a minute scale. The second lemma (l_2) is in all respects a normal leaf with sheath, blade and ligule; that is, the membranaceous scale now extends from side to side across the lamina. On the other hand its claim to be a lemma is equally strong because it encloses a palea (B 2)

and a complete flower (C 2). The third lemma (l_3) has an entire sheath, and on the shoot in its axil a small palea (B 3), but the sexual organs are completely aborted. The remaining two leaves have no indication of flowers in their axils and are perfectly normal though very small foliage leaves. The elongated rhachilla ends in a cluster of seven spikelets which contain from one to three flowers, which although rather small are of quite normal structure. This production of sterile glumes beneath the flowers on the rhachilla shows how completely vegetative it has become. The same phenomenon has been figured for *Festuca ovina* by Jenkin (1921) and for both *Poa laxa* and *P. bulbosa* by Frank (1880), and a possibly parallel occurrence in *Lilium* by Green (1914) where the bulbil bears a terminal flower.

Deschampsia caespitosa Beauv.

This species was investigated in order to determine the part played by the dorsal awn in the formation of the blade, and whether the lateral lobes of the lemma take any part in the formation of the ligule in a grass in which the margin of the ligule is nerved.

Spikelet 1 (Fig. 4A). This spikelet has been only slightly affected by proliferation. The glumes (g) and the first lemma (l_1 and Fig. 4B) are unaltered and the latter has on the shoot in its axil a palea and a perfect flower. In the second lemma (l_2 and Fig. 4C) the awn has become terminal, short and broad and the two inner lobes of the lemma have become narrowed and strongly nerved. The lateral lobes are unaltered. There is a sudden transition to the third lemma (l_3 and Fig. 4D) which is a normal leaf with a true ligule. The gap in the sequence is bridged in the next spikelet to be described.

Spikelet 2 (Fig. 5A). In this spikelet a more advanced state of proliferation is seen. The first lemma (l_1 and Fig. 5B) is elongated and trifid, three nerves entering the median lobe and one each of the lateral lobes; on the shoot in its axil is a palea and a well-developed flower. The second lemma (l_2 and Fig. 5C) encloses a palea but no sexual organs. It is also elongated and trifid but below the apex it shows a constriction; at this level on the ventral surface is an oblong membranaceous scale inserted across the median nerves. The third lemma (l_3 and Fig. 5D) resembles the second in every respect but one, which is that it possesses a true ligule. Comparison of the figures leaves little doubt that this ligule represents the lateral lobes of the lemma joined by the membranaceous scale. The notch between the lateral and median lobes of lemmas similar to l_2 and from the same inflorescence varied considerably in depth, sometimes extending to

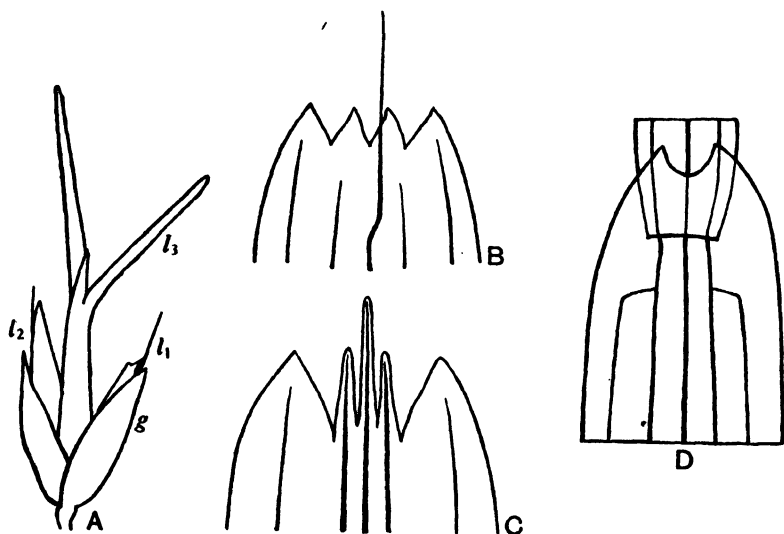


Fig. 4. *Deschampsia caespitosa* Beauv. Proliferating spikelet 1. A, the spikelet, $\times 10$; g , glumes; l_1 - l_3 , successive lemmas. B and C, apices of l_1 and l_3 , $\times 30$. D, ligular region of l_3 , $\times 30$.

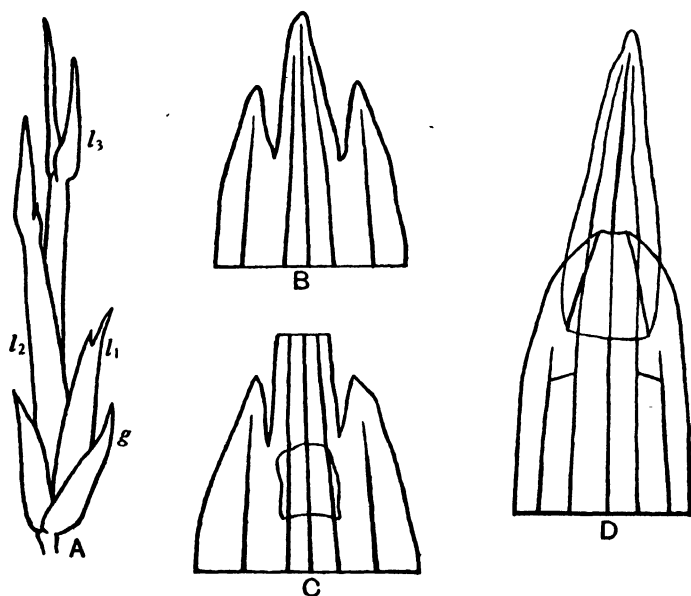


Fig. 5. *Deschampsia caespitosa* Beauv. Proliferating spikelet 2. A, the spikelet, $\times 10$; g , glumes; l_1 - l_3 , successive lemmas. B, apex of l_1 , $\times 30$. C and D, ligular regions of l_1 and l_3 , $\times 30$.

the level of the insertion of the scale and never being quite absent; the figure is of an average example. Even if the notch were absent a comparison of the nervation with Fig. 4D would show that the lateral lobes are included in the ligule in this species.

DISCUSSION

It is evident from these descriptions that on the main axis of proliferated spikelets only the lemmas develop into leaves, the glumes remaining practically unaltered and the paleas when present retaining their characters, though sometimes enlarged or reduced. That the leaves of these proliferated spikelets are equivalent to lemmas is certain, for they frequently have a palea and sexual organs in their axils.

The dorsal awn. In the first spikelet of *Deschampsia* described the dorsal awn is seen to become terminal and to become associated with the two strongly nerved median lobes of the lemma. In the second spikelet this association is complete, minute notches between the three median nerves being found in only one of the spikelets dissected from this inflorescence. That the blade corresponds in this species to the median and terminal part of the lemma is supported by the blades of the smaller leaves of the proliferated spikelets being always three nerved, and by the inclusion of the lateral lobes in the ligule. In *Dactylis*, on the other hand, the entire upper portion of the lemma enters into the formation of the blade. The structure of these spikelets therefore shows that the classical interpretation of the dorsal awn as equivalent to the blade must be abandoned. This hypothesis was open to attack on other grounds, as the insertion of the awn in many species, e.g. *Agrostis canina*, may vary from subterminal to almost basal, and with it presumably would vary the distinction between sheath and ligule, a supposition for which there is no support. It is possible, however, that terminal awns when strongly developed may represent the whole blade. In grasses in which the lemma tapers gradually, the base of the awn may be three nerved, and this is even the case in *Stipa pennata* (Fig. 6A) where the junction of awn and lemma is well defined.

The sheath. The ligule rudiment marking the junction of sheath and blade develops nearer to the apex of the lemma than to the base, so that the lowest part at least of the normal lemma would represent the sheath. In *Deschampsia* practically the whole lemma is equivalent to the sheath of the vegetative leaf, the blade developing by the

elongation of the apical region. It is possible that in *Dactylis* also the blade is only represented by the apex of the lemma. The transition from a lemma with an open sheath to a leaf with the sheath closed is seen to take place gradually and easily in the second spikelet of *Dactylis*; even in normal lemmas the base frequently encircles the rachilla, but it is difficult to imagine how a flower with a closed lemma could open to effect pollination. In *Nardus stricta*, where the anthers

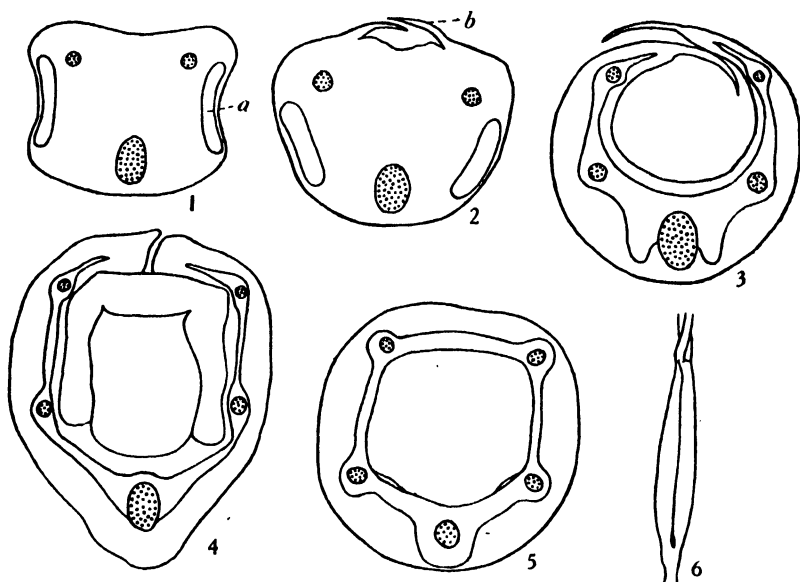


Fig. 6. *Stipa pennata* L. Transverse sections of cleistogamous spikelet. Vascular bundles stippled. 1, 2, 3, junction of awn and lamina: *a*, thin-walled "rotatory" tissue; *b*, margins of lemma; $\times 60$. 4, 5, base of lemma, $\times 60$. 4, lemma open. 5, lemma closed. 6, lemma with basal hairs and most of awn removed, $\times 5$.

are exerted apically and there is no need for the wide opening of the flower, there is a slight basal fusion of the margins of the lemma (Arber, 1928). I find a similar fusion of varying extent in the lemma of *Stipa pennata* (Fig. 6B), where the flowers are cleistogamous. This fusion of the margins where movement of the lemma is of no consequence indicates that the tendency is probably latent in normal grasses. With these considerations before us it seems impossible to accept Bugnon's hypothesis that the sheath is unrepresented in the lemma.

The most plausible view is that a lemma rudiment diverges in its development from a vegetative leaf at a stage previous to the differ-

entiation of the latter into sheath and blade. But these regions are represented potentially in a lemma rudiment as is shown in proliferated spikelets where the lemma rudiment develops into a vegetative leaf.

The ligule. The account of the morphology of the lemma given by von Mohl in 1845 seems to have been overlooked by Bugnon, for in this early paper it is contended that the position of the ligular scale

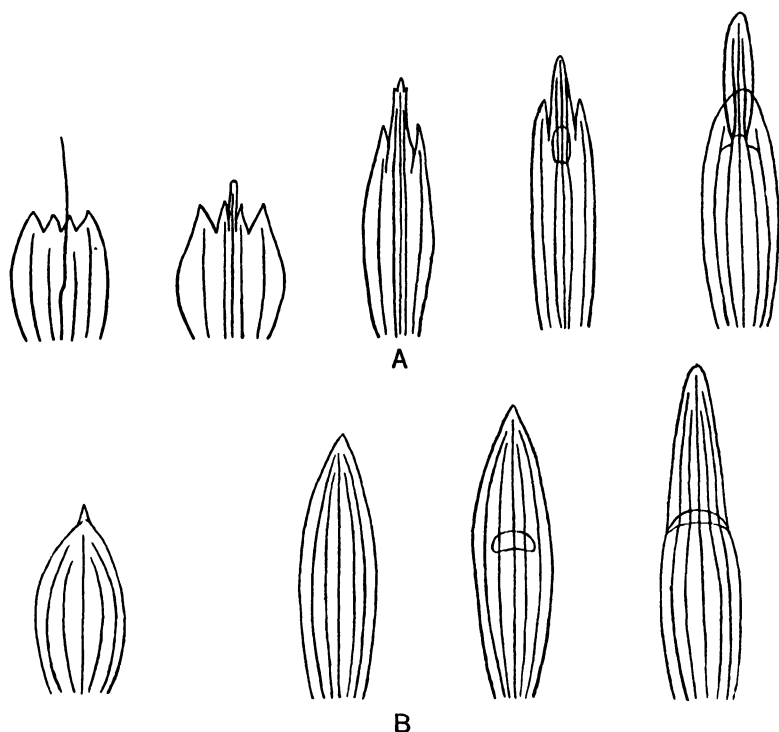


Fig. 7. Diagrams to show the transition from lemma to leaf in A, *Deschampsia alpina* and B, *Dactylis glomerata*.

marking the junction of sheath and blade in the leaves of proliferated spikelets of *Poa alpina* shows that the sheath must be represented in the lemma of this species. The formation of the ligule in the proliferated spikelets of this grass is exactly intermediate between that in *Dactylis* and *Deschampsia*. The transparent margins of the lemma become confined to the lower part of the leaf, their free upper borders eventually becoming connected by the ligular scale, forming the complete ligule. The figures of Stebler and Schröter

(1889) are on a larger scale than those of von Mohl, but these authors seem to have overlooked the membranaceous scale, although they show the formation of the lateral part of the ligule very clearly. In *Deschampsia* the whole of the two lateral lobes each with its nerve become connected in this way, while in *Dactylis* the ligule in proliferated spikelets, as in the normal leaf, consists entirely of the membranaceous upgrowth. These variations in the width of the marginal part of the lemma which corresponds to the lateral parts of the ligule is clearly connected with the question of the morphology of the ligule, a subject on which there has been much difference of opinion.

The types of transition from lemma to leaf shown in the proliferated spikelets of *Dactylis* and *Deschampsia* are set out diagrammatically in Fig. 7, which forms a summary of this paper, and leaves little room for doubt on the three problems of the morphology of the lemma enumerated in the introduction.

I am very grateful to the Director of the Royal Botanic Gardens, Kew, for giving me every facility both in the Herbarium and in the Jodrell Laboratory. I also wish to thank Mr C. E. Hubbard of Kew and Mrs Arber for much valued advice and criticism.

REFERENCES

- ARBER, A. Studies in Gramineae. IV. *Ann. Bot.* **42**, 173. 1928.
 — Studies in Gramineae. V. *Ann. Bot.* **42**, 391. 1928.
 BUGNON, P. *La feuille chez les Graminées*. Thesis, Caen, 1921.
 CELAKOVSKY, L. J. Ueber die Homologien des Grasembryos. *Bot. Ztg.* **9**, 141. 1897.
 DUVAL-JOUE, J. Étude anatomique de l'arête des Graminées. *Mém. de l'Acad. des Sci. et Let. de Montpellier*. 1871.
 EICHLER, A. W. Ueber einige Infloreszenzbulbilen. *Jahrb. bot. Gart. Berlin*, **1**, 171. 1881.
 EVANS, M. G. The life history of Timothy. *U.S. Dept. Agric. Bull.* No. 1450, Washington. 1927.
 EXO, A. *Poa alpina und die Erscheinungen der Viviparie bei ihr*. Diss., Bonn. 1916.
 FORSTER, A. S. Salient features of the problem of bud-scale morphology. *Biol. Rev.* **3**, 2, 123. 1928.
 FRANK, A. B. *Krankheiten der Pflanzen*. 1880.
 GREEN, M. L. Note on anomalous bulbils in a lily. *Ann. Bot.* **28**, 355. 1914.
 HACKEL, E. Gramineae. In Engler and Prantl, *Die Natürlichen Pflanzenfamilien*, **2**, 2, 1. 1887.
 JENKIN, T. J. Notes on vivipary in *Festuca ovina*. *Rept. Bot. Exchange Club*, **6**, 2, 418. 1921.
 MASTERS, M. T. *Vegetable Teratology*. London. 1869.

- PENZIG, O. *Pflanzen-Teratology systematisch geordnet*, 2. Geneva, 1894; Berlin, 1922.
- SCHUSTER, J. Ueber die Morphologie der Grasblüte. *Flora*, **100**, 2, 13. 1910.
- STEBLER, F. G. and SCHRÖTER, C. *Die besten Futterpflanzen*. Bern. 1884 and 1889.
- THOENES, A. Morphologie und Anatomie von *Cynosurus cristatus* und die Erscheinungen der viviparie bei ihm. *Bot. Archiv*, **25**, 284. 1929.
- TOUMEY, J. W. Peculiar forms of proliferation in Timothy. *Bot. Gaz.* **16**, 12, 346. 1891.
- TURESSON, G. Studien über *Festuca ovina*, L. I. *Hereditas*, **8**, 161. 1926.
- Studien über *Festuca ovina*, L. II. *Hereditas*, **13**, 177. 1930.
- TURPIN, P. F. J. Mém. sur l'inflorescence des Gram. et Cyp. *Mém. du Mus. d'Hist. Nat. Paris*, **5**, 426-92. 1819.
- VAN TEIGHEN, PH. Sur le Cotyledons des Graminées. *Ann. des Sci. Nat.* 5^e sér. **15**, 236. 1872.
- VON MOHL, H. Ueber die Bedeutung der untern Blumenspelze der Gräser. *Bot. Ztg*, **3**, 3, 33. 1845.
- VUILLEMIN, P. *Les Anomalies végétales*. Paris. 1926.

SOME CRITICAL EXPERIMENTS UPON CULTURE METHODS USED FOR FUNGI

By J. L. HARLEY

Department of Botany, Oxford

(With 11 figures in the text)

I. INTRODUCTION

IN the field of morphological investigation where it was first evolved the Petri-dish technique has no peer as far as rapidity of manipulation of large numbers of cultures is concerned. This very fact has led to its being used for physiological investigations, firstly of the factors necessary for the development of morphological structures within the culture and later of the factors, both chemical and physical, which effect the sequence of events so difficult to estimate, termed growth. The tacit assumption has been generally made that the cultures are reared under controlled conditions, and that justifiable comparisons can be obtained under different treatments when this technique has been used. The work on the staling of cultures has done something to dispel this misconception, but staling has, in the main, been connected with old age of the culture alone, and the early stages of growth are frequently believed to be free of that factor, or that set of factors, which finally brings about death in old cultures.

That some change of hydrogen-ion concentration occurs in fungal cultures during growth has been known since the work of Nikitinsky on mould fungi in 1901; this factor was invoked by Brown (1922) to explain alkaline staling, and later by Pratt (1924) and Marloth (1931) to explain the increase of carbonates and bicarbonates in alkaline staling cultures. In spite of the prominence into which the above workers, especially Brown, brought the subject of staling, many observers believe that staling is a property of relatively few fungi. In actual fact, however, the literature shows that all fungi in which the point has been investigated change the hydrogen-ion concentration of the medium to a greater or less degree. In the face of these observations the possibility of obtaining reliable results from work with Petri-dish cultures diminishes considerably, especially as it is generally held that pH may act as a direct factor on the rate of growth. In the

following experiments therefore the march of pH change in cultures together with the rate of growth have been measured with a view to emphasising the limitations of the technique.

II. EXPERIMENTAL

Preliminary experiments

In the preliminary experiments the fungi used were found to fall into two groups:

(a) Those in which the direction and extent of the pH change depended mainly upon the nitrogen source presented in the culture medium.

(b) Those in which the change was always towards the acid side and the extent of the change only depends on the nitrogen source.

In the first group were: *Neocosmospora vasinfecta*, *Chaetomium pannosum*, *Sordaria fimicola*, *Alternaria citri*. In the second was *Sclerotinia sclerotiorum*. The following table illustrates this point.

Nitrogen source	<i>Neocosmospora</i>			<i>Sclerotinia</i>	
	Initial pH	Final pH		Initial pH	Final pH
		6 days	12 days		
Potassium nitrate	5.0	7.2	8.4	6.8	3.9
Calcium nitrate	—	—	—	5.6	2.8
Ammonium nitrate	4.9	3.2	16 days	6.8	3.5
			2.8		
Ammonium sulphate	4.6	2.8	16 days 2.4	6.8	2.8
Ammonium tartrate	—	—	—	6.8	4.2

The change of pH caused by *Neocosmospora* upon medium containing potassium nitrate is similar to that sequence of events which was investigated by Pratt as alkaline staling. The nitrate is more rapidly absorbed than the potassium, resulting in free hydroxyl ions being left in the culture. In the presence of ammonium salts, this fungus causes a change to acid values. This is not so extensive, however, in ammonium nitrate culture. These observations are amply explained by the rapid absorption of ammonia leading to the release of free hydrions. The change is partly counteracted when nitrate is also present and is absorbed.

In cultures of *Sclerotinia* change towards acid values is counteracted by the absorption of nitrates and increased by the absorption of ammonia. In the case of ammonium tartrate, however, it is probable that the acid radicle acts as source of carbon; and in the case of

calcium nitrate the calcium probably precipitates acid produced with a release of more highly ionised nitric acid.

In the main body of the experiments *Neocosmospora* only was used, as very equal and circular cultures of this fungus can be obtained easily by using fairly immature perithecia with their attached hyphae as inocula. The first point brought out was that the culture medium could not be assumed as homogeneous as regards hydrogen-ion concentration at any time after the fungus had begun to grow. Thus by cutting concentric rings of medium from a culture, a gradient of pH was discovered from the centre outwards.

In a culture of *Neocosmospora* 51 mm. in diameter, growing on a plate of Richards' agar 90 mm. in diameter of initial pH 5.0, the following gradient was established from the centre outwards:

Zone (mm.)	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90
pH	9.6+	9.6+	9.6	9.6	8.3	7.5	6.6	5.8	5.8

Similarly in a culture 29 mm. in diameter the gradient was:

Zone (mm.)	0-10	10-15	15-25	25-30	30-40
pH	7.0	6.6	6.2	6.0	5.5

The other fungi showed similar gradients, e.g. *Chaetomium* culture 40 mm. diameter:

Zone (mm.)	0-15	15-25	25-30	30-35	35-40
pH	6.0	5.8	5.7	5.6	5.6

The pH values were tested by a colorimetric ("capillator") method which necessitated melting of the medium, but the errors due to displacement of carbon dioxide in manipulation were shown to be negligible at pH values up to 8.5. Above that the heating caused rises in pH value. However, the figures establish the presence of a definite gradient. The explanation of this lies in the fact that in agar all movement of solutes must be by diffusion alone in the absence of convection currents. The hydrogen and hydroxyl ions are well known to have a greater diffusion rate than other ions present, so that gradients of all food substances and products absorbed and produced are certainly present in the medium. The steepness of these gradients must depend upon rate of absorption or release, initial concentration, and upon the size of the diffusing particle. In the case of starch as source of carbon the gradient of movement of enzyme outwards and starch inwards is clearly shown by staining plates with iodine when concentric rings of colourless, red, purple, blue and black colours are produced about the fungus.

For the purpose of correlating pH change and increase of diameter of a colony it seems probable that the pH value of the marginal zone of the fungus will prove most valuable. Here the elongation of the hyphae takes place, and as the culture ages the onus of food absorption falls more and more upon this zone, as the central regions of the culture are exhausted of food substance which is replaced very slowly by diffusion. The pH of the medium round the relatively unbranched

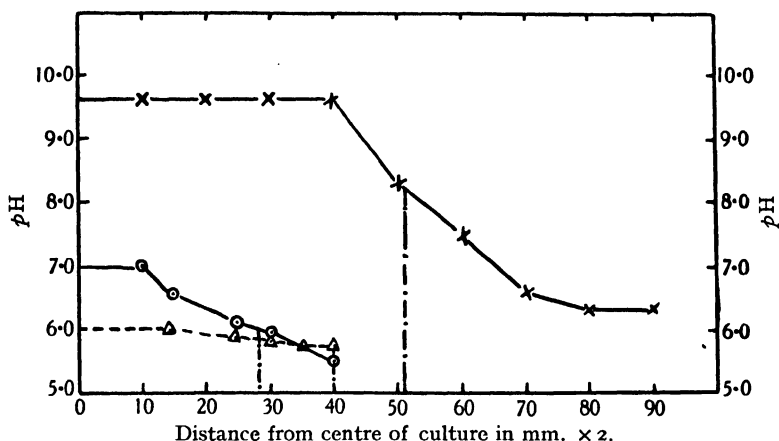


Fig. 1. The hydrogen-ion concentrations at various points in Richards' agar culture medium plotted against distance in millimetres from the centre of the culture. \times — \times *Neocosmospora* 51 mm. in diameter. \odot — \odot A culture of the same 20 mm. diameter. \triangle — \triangle *Chaetomium* 40 mm. diameter. The broken vertical lines show the limit of the colonies upon the plates.

marginal zone, has therefore to be used throughout as the pH value of the culture for comparison with the rate of outward movement of the margin.

Nitrate cultures

Fig. 2 shows the results of an experiment in which *Neocosmospora* was grown upon Richards' agar of initial pH 5.0. The daily increment curve is derived from an average of forty-seven cultures which were diminished in number daily to provide cultures for testing the concurrent pH change shown on the broken curve. The acceleration of growth rate is great at first as shown by the rising curve, but gradually diminishes so that successive daily increments become approximately constant, and later after seven days a gradual diminution of increment begins and increases up to the twelfth day when the experiment was discontinued. The pH during this time rises from 5.0 to 8.5, the final falling phase of growth rate beginning about 7.2.

Over this change of pH many subsidiary effects must be present. Firstly it entails the change of the acid phosphate of potassium present in the original medium, through neutral phosphate to alkali phosphate. This change itself buffers the medium greatly and in this zone indeed the buffering of the medium is at its greatest. Two changes take place after the medium has reached alkalinity. Firstly carbon dioxide becomes soluble and the free hydroxyl ions are replaced partly by carbonate ions resulting in a secondary buffering of the medium. Again magnesium sulphate and potassium phosphate in

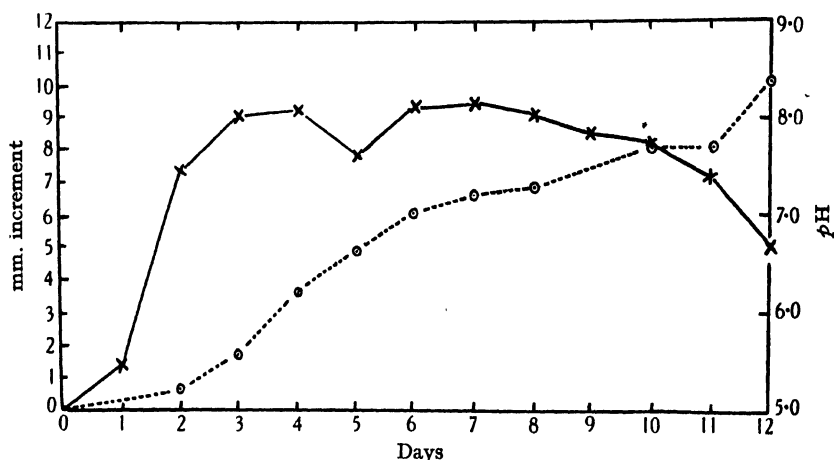


Fig. 2. *Neocosmospora* upon nitrate agar (Richards'). Daily increments in diameter measured in millimetres are plotted against time in days, $\times - \times$. The broken curve shows the pH of the marginal region of the culture at time intervals corresponding to diameter measurements.

the presence of alkali give rise to an insoluble magnesium-potassium double phosphate which is precipitated. Thus these elements are removed partly or wholly from solution.

It may be demonstrated that the removal of magnesium by this means has little effect upon the growth of *Neocosmospora*, since growth is easily possible when magnesium is not added to the medium. Good growth, similar to that produced on normal Richards' medium, is produced on medium in which the only magnesium present is that contained by the agar as an impurity. The curves of an experiment of this sort are shown in Fig. 3 and are of the same general form as those of Fig. 2. In this case the phosphate and the potassium are not precipitated, so that the absence of these cannot be a contributory cause either of the establishment of the phase of constant growth

rate or the subsequent diminution of growth rate. The standardisation of normal media, however, to pH values above neutrality results always in the precipitation of this double phosphate and in the complete removal of magnesium when large quantities of alkali are added. The precipitation in the presence of agar is slow and the buffer effect is gradual. This fact is overlooked by many workers on hydrogen-ion concentration, and has resulted in considerable errors of standardisation as well as in the presentation of media of different constitution to the fungus at the alkaline pH values. This difference of constitution

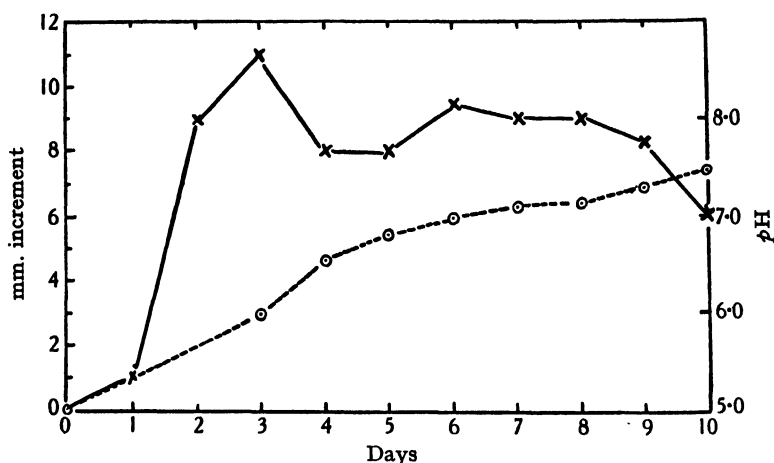


Fig. 3. *Neocosmospora* upon nitrate agar (Richards') without magnesium. Daily increment curve, $\times-\times$. pH values, the marginal zone of the colony, $\odot-\odot$.

seems to be of little importance in the case of *Neocosmospora*, but how far the demands of other fungi for these ions coincide with those of the latter is unknown. At any rate the point requires attention.

There are left three possible factors which may be contributory to the establishment of the level and falling phases of growth rate:

- (1) Change of pH to inhibitory values.
- (2) Production of carbonates and bicarbonates.
- (3) Removal of food substance and the limiting of processes by diffusion rate.

The first two factors may be eliminated by the growth of the fungus upon medium standardised at 3.2 initially. Here a totally new region of pH is presented to the fungus for the first part of its growth, and at pH values below neutrality no carbonates and bicarbonates are produced in culture.

The results are shown in Fig. 4. The first stages are characterised by a very slow rate of growth which increases as the pH rises. The acceleration, however, does not increase, but drops away, although the pH is rising to more optimal values. It must be assumed, therefore, that the exhaustion of food substances must be counteracting any increase of rate brought about by pH change. In this experiment the pH change is more rapid than before because the initial pH is below the zone of maximum buffering by the phosphates. Above 7.2 the fall of growth rate again begins.

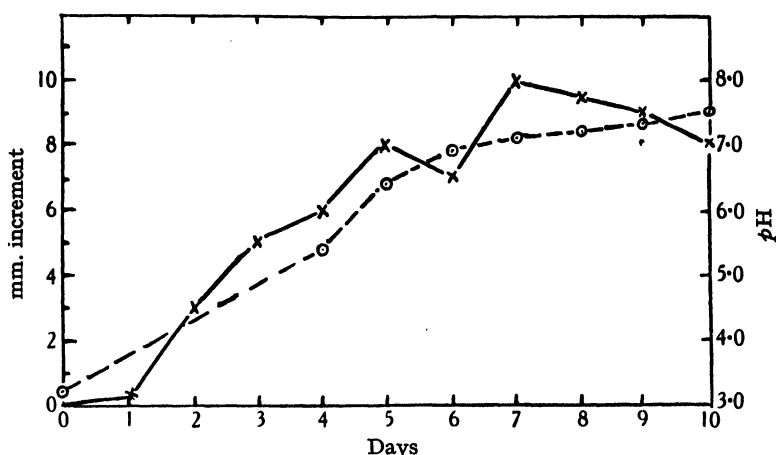


Fig. 4. *Neocosmospora* upon nitrate agar (Richards') initially standardised to pH 3.3. The daily increments of diameter are shown on the unbroken curve and the pH about the marginal zone of the fungus at daily intervals is shown on the broken curve.

The probable sequence of events in these experiments may be summarised then as follows: At first the rate of growth is dependent upon the surface of the hyphae of the colony and it increases as this surface increases. Later the exhaustion of the medium about the colony results in the limitation of growth by the slow rate of diffusion of food substances that follows, leading to a level phase where this is the dominant factor controlling growth. Later the effect of pH change to alkali values coupled with carbonate and bicarbonate effects results in the fall in growth rate known as staling.

Ammonium cultures

On media containing ammonium as nitrogen source the sequence of events is entirely different. Fig. 5 shows an experiment in which *Neocosmospora* is grown on Richards' agar in which ammonium sul-

phate was substituted for potassium nitrate so that the total nitrogen remained the same, potassium being still present as phosphate. The initial stages of growth were comparable with those on nitrate media showing an increase in growth rate; later the growth rate fell rapidly from the third day, when it was 8 mm. a day, to nothing after seven days. During this time the pH changed rapidly to acid, values becoming 2.8 on the seventh day and ending at 2.4 on the sixteenth day. On this range the buffering of the medium is slight. On the fifth day several plates were treated with alkali and the pH raised to 3.9. These plates showed a growth of 5 mm. for the subsequent 24-hour

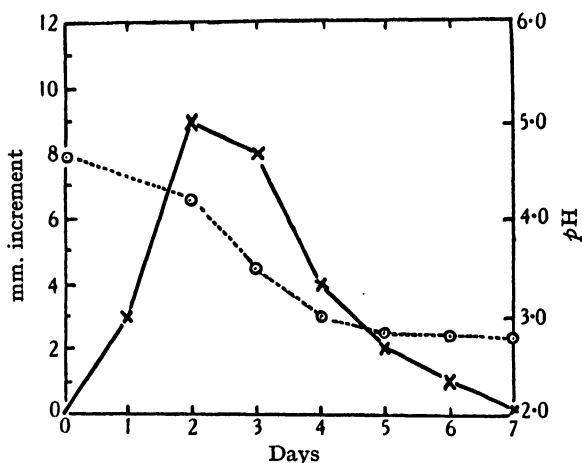


Fig. 5. *Neocosmospora* upon ammonium sulphate agar (modified Richards'). Daily increment in diameter plotted against time on the unbroken curve and pH of the marginal zone of the fungus on the broken curve.

period as compared with 1 mm. for untreated plates whose pH was 2.9. After sixteen days no such increase was observed and the cultures were assumed to be dead.

This was repeated on a medium in which the initial pH was raised to 6.8. The growth rate increased to a maximum which was maintained for nine days during which the pH fell slowly in this heavily buffered region to 5.8 (Fig. 6).

These facts show that the ammonium ion provides an effective source of nitrogen for *Neocosmospora*, and that at pH values from neutrality to 5.0 at any rate it is capable of supporting successful growth. The release of hydrogen ions caused by its absorption, however, bring about low pH values of the medium which result in decrease of growth rate.

When the nitrogen is supplied as ammonium nitrate, the ammonium ion is absorbed preferentially as is shown by the change of

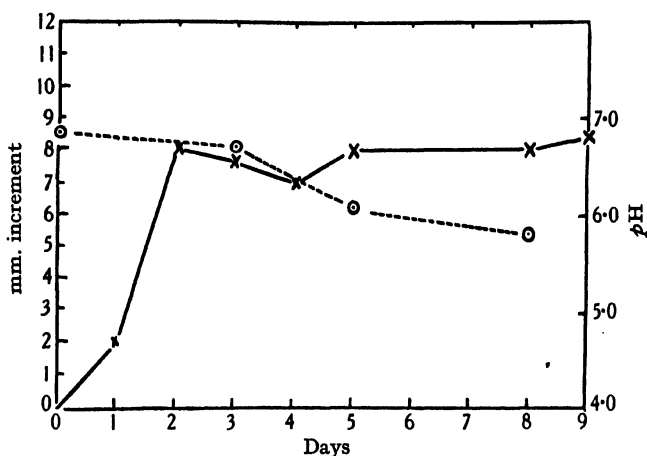


Fig. 6. *Neocosmospora* upon ammonium sulphate agar (modified Richards') initially standardised to pH 6.8. The unbroken curve shows daily increment in diameter plotted against time. The broken curve shows the pH of the marginal zone of the fungus at various time intervals.

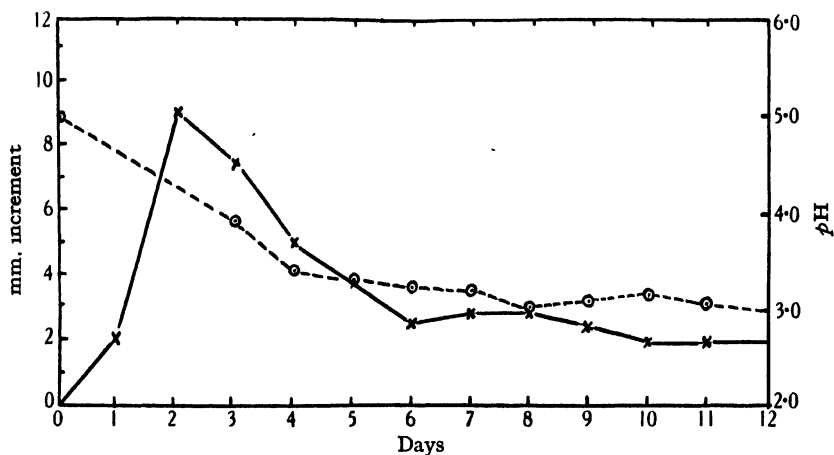


Fig. 7. *Neocosmospora* upon ammonium nitrate agar (modified Richards'). The daily increment of diameter of the fungus is plotted against time in days on the unbroken curve, whilst the broken curve shows the pH of the marginal zone of the colony at given time intervals.

pH towards acid. The results of such an experiment are shown in Fig. 7. The curve of daily increment of growth against time is of the same form as that on ammonium sulphate medium, yet the change

of pH and consequently the fall of growth rate is slower. The effect of the nitrate present is to raise the pH of the medium of the central region of the culture where it is absorbed after the ammonia is exhausted, whereas in cultures containing ammonium sulphate only the central region of the culture is invariably more acid than the margin.

This is shown by the following figures:

	Age days	pH of central region	pH margin
NH_4SO_4	6	2.4	2.8
NH_4NO_3	6	4.4	3.2
NH_4NO_3	9	3.7	3.1

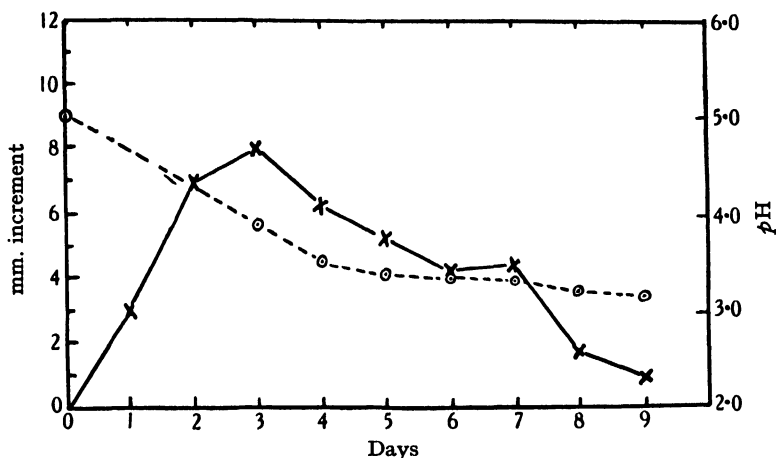


Fig. 8. *Neocosmospora* upon ammonium sulphate and potassium nitrate agar in which the nitrate is present as three-quarters of the total nitrogen. The daily increment of growth is shown in the unbroken curve and the pH on the broken curve.

Even in cultures where the nitrogen is present mainly as nitrate and only partly as ammonium ions the hydrogen-ion concentration falls to acid values during growth. Fig. 8 shows the increment and pH change curves for cultures containing a quarter of the nitrogen as ammonia. It will be seen that there is still a decrease in growth rate coupled with a fall of pH in the marginal zone of the fungus.

Other changes are coupled with the decreased growth in diameter at low pH values. The medium about the fungus becomes red due to the diffusion outwards of some coloured metabolic product. When extracted from the medium by butyl alcohol, it turns blue on addition of alkali, gives a blue-violet colour with sodium carbonate, and with

ferric chloride gives a greenish blue coloration. The colour change caused by the first two reagents is reversible on treatment with dilute hydrochloric acid. It is therefore possibly the anthocyanin pigment liberated during abnormal metabolism at high acidities.

Some of these experiments were repeated with liquid cultures of *Neocosmospora*. The growth rate was estimated by dry weight increase. Here, however, the movement of ions by convection in solution is possible, and the change of ionic concentration brought about by uptake and release of ions by the fungus is by no means so localised as in the agar cultures. The results were substantially the

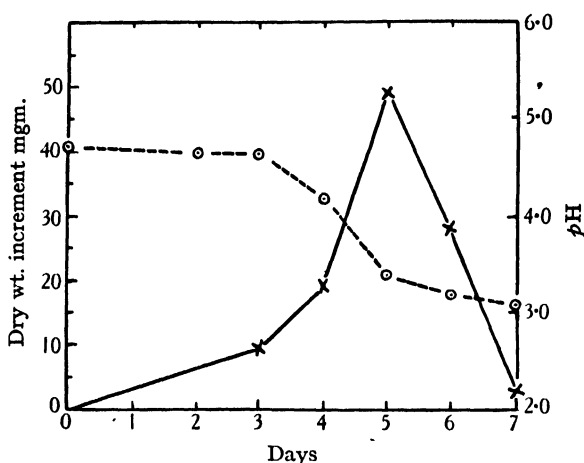


Fig. 9. *Neocosmospora* in liquid medium containing ammonium sulphate of initial pH 4.6. The dry weight increment plotted against time on the unbroken curve, and the pH of the medium against time on the broken curve.

same. Water cultures with NH_4SO_4 as nitrogen source and of initial pH 4.8 showed a rapid rise of dry weight increment up to the fifth day and then a fall to the seventh day. The pH fell from 4.8 to 3.0 during this time and later fell further to 2.4 (Fig. 9). Other similar cultures standardised to 6.8 initially showed a rise in increment daily up to seven days, whilst the pH fell to 4.8 (Fig. 10). No flat-topped curve comparable with those of growth on agar was found. This is due it is suggested to the fact that supply of nutrients is never limited by diffusion rate and that it is presented to the whole fungus body for absorption and not mainly to a restricted marginal zone.

In liquid cultures analysis of sugar uptake was possible, and the glucose in modified Richards' medium was estimated by Hanes'

modification of the Hagedorn-Jensen sugar titration (Hanes, 1929). For this purpose 1 c.c. cultures of media were inoculated and the

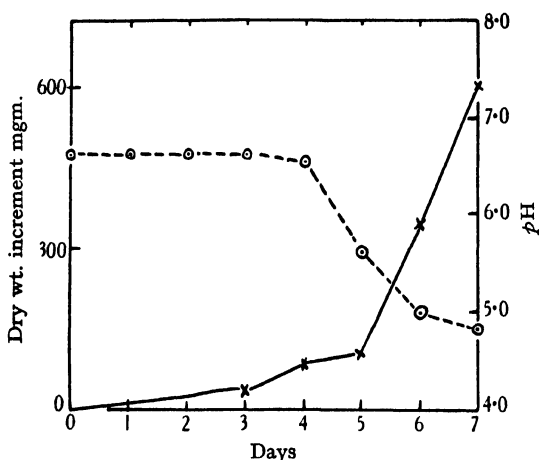


Fig. 10. *Neocosmospora* in liquid medium containing ammonium sulphate. The initial pH was standardised to 6.6. The dry weight increment is plotted against time on the unbroken curve and the pH of the medium against time on the broken curve.

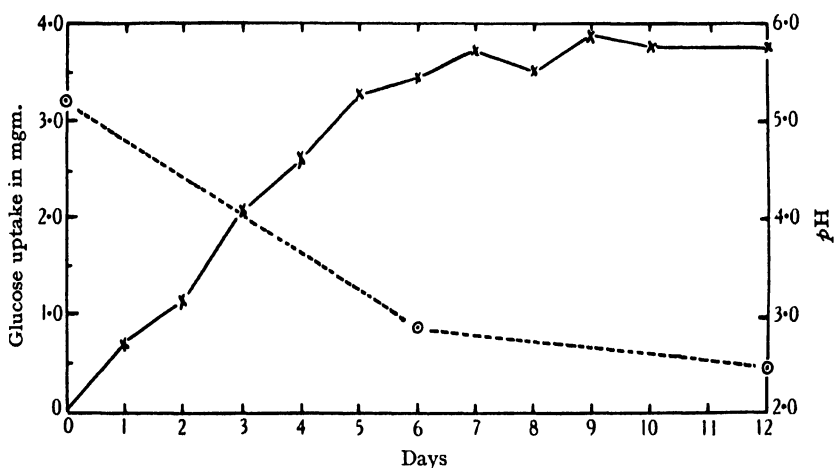


Fig. 11. *Neocosmospora* in 1 c.c. culture of modified Richards' liquid medium. The total glucose uptake is shown against time on the unbroken curve and the pH of the medium on the broken curve.

changes of pH and concentration were rapid but in no case was the sugar nearly exhausted.

*Sugar uptake in cultures of Neocosmospora on
glucose and $(\text{NH}_4)_2\text{SO}_4$ media*

Days	Sugar present mgrm.	Amount taken up mgrm.	pH
0	22.2	—	5.2
2	21.5	0.7	—
3	21.0	1.2	—
4	20.2	2.0	—
5	19.5	2.7	—
6	18.9	3.3	2.8
7	18.7	3.5	—
8	18.4	3.8	—
9	18.6	3.6	—
10	18.3	3.9	—
13	18.4	3.8	2.4

The sugar uptake falls off as high acidities are reached in the medium and parallels the growth rates as estimated by dry weight (see Fig. 11).

III. SUMMARY

These experiments bring out certain limitations in the technique used in the culture of fungi which may be summarised as follows:

(1) The absorption and release of substances by fungi during growth materially changes the constitution of the media presented to them.

(2) The exchange is limited in agar cultures by diffusion and this may affect the rate of growth markedly. The establishment of a gradient of hydrogen-ion concentration from the centre of a culture in agar, extending outwards into the medium beyond, forces the deduction that a similar gradient exists for less diffusible ions. The medium is therefore not homogeneous. The pH of the margin of the colony is most likely to have a direct effect on growth rate.

(3) Changes to alkalinity due to nitrate absorption bring in their train subsidiary changes:

- (a) Precipitation of phosphate, potassium and magnesium.
- (b) Removal of carbon dioxide into solution with formation of carbonates and bicarbonates.

(4) Changes to acidity may be due to:

- (a) Ammonia absorption (as by *Neocosmospora*).
- (b) Release of acid metabolic products (as by *Sclerotinia*).

(5) Sugar uptake is inhibited by high acidities developed in *Neocosmospora* cultures upon ammonium salts in liquid media.

In conclusion certain other limitations may be emphasised. The effects of addition of any substances to the medium whose solubility is

changed by the acidity of the medium cannot be investigated by these methods. Thus, for instance, problems of gas storage dealing with the use of CO_2 , SO_2 , H_2S , etc., must be done with a method in which both the gas stream and the medium may be periodically renewed.

Again, the addition of agar, containing as it does hexoses, pentosans, pectic substances and metallic elements, invalidates chemical work on the physiology of fungi. Also, as has been pointed out by Smith (1932), changes of constitution, especially with regard to sugars of the medium, on heat sterilisation, are likely to lead to false assumptions of the constitution of the media presented to the fungus.

Lastly, the conditions of aeration are uncontrolled in most methods of culture. Data have been collected on this point, but are not quoted above. The reader is referred to the work of Bennet-Clark published recently in this Journal.

The writer wishes to express his thanks to Mr W. H. Wilkins, under whose general supervision this work was done.

REFERENCES

- BENNET-CLARK, T. A. Rôle of organic acids in plant metabolism, III. *New Phyt.* v. **32**. 1933.
- BROWN, W. On the growth and germination of fungi at various temperatures and in various concentrations of oxygen and carbon dioxide. *Ann. Bot.* **36**. 1922.
- Some experiments on the growth of certain fungi in culture media. *Ann. Bot.* **37**. 1923.
- HANES, C. Application of the Hagedorn-Jensen method to the determination of large quantities of reducing sugars. *Biochem. J.* 1929.
- MARLOTH, R. H. Hydrogen-ion concentration studies on *Penecillium italicum* and *Penecillium digitatum*. *Phytopath.* **21**. 1931.
- NIKITINSKY, J. Ueber die Beeinflussung der Entwicklung einige Schimmelpilze durch ihre Stoffwechselprodukte. *Jarb. Wiss. Bot.* **40**. 1904.
- PRATT, C. Staling of fungal cultures. I. *Ann. Bot.* **38**. 1924.
- Staling of fungal cultures. II. *Ann. Bot.* **38**. 1924.
- SMITH, M. L. Effect of heat on sugar solutions used for culture media. *Biochem. J.* **26**. 1932.

BASIC CHROMOSOME NUMBERS IN PLANTS WITH SPECIAL REFERENCE TO THE COMPOSITAE

By ERNEST B. BABCOCK

University of California, Berkeley

AN attempt has been made by Wanscher (1934) to derive important conclusions concerning the basic chromosome number of the higher plants from a statistical study of the frequency of occurrence of chromosome numbers in forty-four families represented in the list published by Tischler in 1931. The present writer maintains that this effort has failed to produce results of any general significance. That such an effort must be futile would seem obvious. In the first place Tischler's list is far from being a complete summary of previously published reports on chromosome numbers. Secondly, even if this list had been complete, it would include only a very small fraction of the species in the families represented. Thirdly, in the opinion of the present writer statistical analysis of the distribution of chromosome numbers by itself is inadequate for the determination of phyletically basic chromosome numbers.

The last statement is based on the results of extensive research on chromosomes and phylogeny in *Crepis*. Nearly half of the species in this genus have been examined cytologically and these represent all subdivisions of the genus. It happens that the most prevalent haploid chromosome number in *Crepis* is 4, fully one-half of the one hundred and seven species hitherto counted having this number, while less than one-fifth have 5 pairs of chromosomes. Nevertheless, 5 must be the basic chromosome number in *Crepis* for the following reasons: (1) The most primitive species, as determined by comparative morphology, in all three subgenera have $n = 5$. (2) The most recent species in the genus, as indicated by extreme reduction of the plant and specialisation of certain parts, have $n = 4$. (3) The morphology of the chromosomes themselves points to the same conclusion. There are five types of chromosomes in this genus which have been designated by Navashin (1925) as *A*, *B*, *C*, *D*, *E*. Now all of the 5-paired species have all five of these chromosome types, while none of the 4-paired species, or the 8-paired polyploids derived therefrom, has the *E* chromosome. Finally, all other chromosome numbers

in *Crepis* have been shown by Babcock and Cameron (1934) to be secondary and derived directly or indirectly from the basic number, 5. Thus the published statements of various authors that because 4 is the most prevalent chromosome number in *Crepis*, it must be the most basic number, are erroneous. On the other hand, it may turn out in other genera or other families that the most prevalent chromosome number is also the most basic. But, in view of the situation in *Crepis*, it is clear that inferences concerning basic chromosome numbers should not be drawn from the results of statistical study alone. Of much greater importance is the consideration of all available evidence, especially that from comparative morphology and cytogenetics.

The futility of the statistical method when applied to inadequate data is well illustrated by the situation in the Compositae when other data on species of this family which were published before 1931 are taken into account. For sake of simplicity we may consider only three lower numbers in the two series with lowest base numbers. The number of species falling in one or other of these two series as based on Tischler's list is compared below with the number of species available in other literature combined with those in Tischler's list.

	Haploid nos.			Total	Haploid nos.			Total
	4	8	16		5	10	20	
Tischler, 1931	33	8	4	45	13	—	2	15
In literature previous to 1931	39	23	6	68	23	20	29	72

From these data it appears that the number of species in the Compositae in the 5-10-20 series is larger than the number in the 4-8-16 series. But possibly when the chromosomes are counted in many more species of this family the situation will be greatly changed. From the data available in 1931, however, it appears just as likely that the basic number is 5 as that it is 4. No doubt similar situations would be found in some of the other families represented in Tischler's list, if all available data on chromosome numbers were considered.

That chromosome number alone is an inadequate criterion of relationship was made obvious by the comparative studies of Heitz (1925-6) on number, size and form of the chromosomes in the plant kingdom. These studies have also brought to light some surprising dissimilarities in size of the chromosomes of related species in such genera as *Cyclamen*. In general, however, there seems to be close similarity in chromosome morphology between the species of natural

genera. This is certainly the case in *Crepis* (Babcock and Cameron, *loc. cit.*). But all these aspects of the chromosomes must be studied in relation to comparative morphology of the plants themselves, while geographic distribution, comparative genetics and cytogenetics will throw further light on phylogeny. The point to be emphasised in the present discussion is that a mere study of the distribution of chromosome numbers in a small fraction of the species in a family or genus, and especially without due consideration of other evidence on taxonomic relationships in those groups, may lead to quite erroneous conclusions concerning basic chromosome numbers.

REFERENCES

- BABCOCK, E. B. and CAMERON, D. R. Chromosomes and phylogeny in *Crepis*. II. The relations of one hundred and nine species. *Univ. Calif. Pub. Agr. Sci.* 6 (in press).
 HEITZ, E. Der Nachweis der Chromosomen. Vergleichende Studien ueber ihre Zahl, Grösse und Form im Pflanzenreich. I. *Zeit. Bot.* 18, 625-81. 1925-6.
 NAVASHIN, M. Morphologische Kernstudien der *Crepis*-arten in Bezug auf die Artbildung. *Zeit. Zellforsch. mikrosk. Anat.* 2, 98-111. 1925.
 TISCHLER, G. Pflanzliche Chromosomen-zahlen. *Tabulae Biol.* 7, 109-226. 1931.
 WANSCHER, J. H. The basic chromosome number of the higher plants. *New Phyt.* 33, 101-26. 1934.

*References to chromosome counts for species of Compositae
 supplementing Tischler's 1931 list*

- AFZELIUS, K. *Acti Horti Bergiani*, 8, 123-219. 1924.
 BELLING, J. *Genetics*, 10, 59-71. 1925.
 BOENICKE, Ber. *Deut. Bot. Ges.* 29, 59-65. 1911.
 BORGSTAM, E. *Arkiv f. Bot.* 17, 1-27. 1922.
 HOLMGREN, I. *Svensk Vet. Akad. Handl.* 59, 118 pp. 1919.
 ISHIKAWA, M. *Bot. Mag. Tokio*, 25, 1-8. 1911.
 — *Bot. Mag. Tokio*, 30, 404-48. 1916.
 — *Bot. Mag. Tokio*, 35, 153-9. 1921.
 LANGLET, O. F. I. *Svensk. Bot. Tidskr.* 19, 215. 1925.
 MARCHAL, Ém. *Mém. Acad. R. Belgique Cl. Scienc.*, ser. 2, 4, 108 pp. 1920.
 OSAWA, J. *Arch. Zellforsch.* 10, 450-69. 1913.
 ROSENBERG, O. *Flora*, 93, 251-9. 1904.
 — *Svensk Bot. Tidskr.* 6, 915-19. 1912.

REVIEWS

Die Flechten. Von FRIEDRICH TOBLER. 9 × 6½ in. Pp. 84. 66 figs. Jena: G. Fischer. 1934. Rm. 5.50.

The Lichen Symbiosis, discovered by Schwendener in 1869, has been interpreted in many different ways since that year. It has been looked upon as a form of parasitism of alga on fungus, of fungus on alga, and as a form of reciprocal but antagonistic parasitism of the two different organisms concerned. It has also been looked upon as an example of benevolent mutualism. In a way most of these interpretations tend to be one-sided and their authors are apt to lay insufficient stress on the finished product of this close association of fungus and alga, the lichen, and rather more on its dual nature. As long ago as 1880 Reinke in his text-book of botany described the relationship met with in the lichens as a consortium. The lichen is a morphological unity, and he wished to indicate that from this close union a new single organism had emerged, the physiological behaviour of which was due to the combined functioning of alga and fungus. Other biologists have expressed similar views. The Moreaus, however, view the lichen practically as a gall caused by a fungus being attacked by an alga. This theory of the relationship of alga and fungus in the lichen emphasises in the strongest possible manner the antagonism to one another of the two organisms concerned. In 1931 Prof. F. Tobler delivered at the invitation of the University of London three lectures on lichens. The substance of these lectures forms the basis of the small pamphlet now under review. It consists of four chapters. The Introduction is followed by a chapter dealing with the nature and life of the lichen. In the third chapter its metabolism and growth are discussed, and in the last chapter the dual nature of the lichen.

Prof. Tobler puts forward very clearly his views regarding the nature of the lichen symbiosis. It is a unity dependent on, and an expression of, the balance of the physiological activities of alga and fungus established by the closest union of these organisms. He brings forward evidence in support of this view. The reviewer has himself not so long ago and almost in the same words put forward and stressed the same idea of the unity of the lichen organism. During the last few years several lichenologists have carried out experiments with the pure culture of the fungal symbiont of *Xanthoria parietina*. All agreed that the alga-less mycelium thus produced exhibited in its structure a slight indication at any rate of the structural differentiation met with in the lichen *Xanthoria parietina*. This interesting discovery does not minimise necessarily the influence of the gonidia on the development of the lichen as a whole. But it does show that the fungal symbiont develops a certain amount of lichen activity itself even when not under the influence of the alga. Prof. Tobler in this connection brings forward again the so-called "pushing hyphae" of Frank and Nienburg described for the protothallus of *Pertusaria*, though the explanation of their activity given by these two authors has been adversely criticised. The close union of alga and fungus brings about a complete change in the metabolism of the fungus and the result must be a complete balancing in the perfect lichen of the physiological activities of the two symbionts. It is owing to this that Prof. Tobler lays so much stress on the idea of the unity of the perfect lichen. Both alga and fungus take an active part in the shaping of the lichen. This point of view Prof. Tobler has worked out very fully. In his concluding remarks he says that the dual origin of the lichens is no longer their main characteristic but their true nature lies in their unity. He leads up to this statement logically, clearly and insistently, and he is thus expressing a view which is more in accord with modern ideas. For that reason his pamphlet should be read with interest by botanists and biologists. Some lichenologists might conceivably consider that he has not made sufficient reference to the recent work of one or two

investigators. But Prof. Tobler intended to bring out mainly his own views and those of his school. That he has succeeded in doing. The illustrations in the pamphlet are on the whole good, but Figs. 12 *a* and *b* might very well have been dispensed with. They are misleading. On p. 15 the word "existenzfähig" should obviously be replaced by "existenzunfähig." The reviewer does not think that the remarks on p. 30 concerning the wide distribution of breathing pores in large lichens are at present quite justified. Much work still remains to be done on the subject of pure lichen anatomy in its physiological aspect.

O. V. DARBISHIRE.

Plant Chimaeras and Graft Hybrids. By W. NEILSON JONES, Hildred Carlile Professor, University of London. Edited by G. R. DE BEER, M.A., D.Sc., for Methuen's Monographs on Biological Subjects. With 21 text-figures. London: Methuen. 1934. 3s. 6d.

The chimaeral structure of plants has become so important an interpretation of certain forms of variegation and of some other perplexing phenomena of plant life quite apart from the comparatively few but interesting graft hybrids, that a short monograph on the subject such as that prepared by Prof. Neilson Jones will be generally welcomed by botanical students and indeed by such members of the general public as are interested in plant life. That the subject-matter of this volume is based upon a course of intercollegiate lectures given to advanced students in the University of London has led the author to arrange his material in an educationally progressive series of chapters, and by judicious notes and references to the most important original memoirs to direct the attention of students to the sources from which they can obtain further and more detailed information. The style is clear, so that the argument is easy to follow and this is facilitated by a well-chosen series of illustrations.

As regards the so-called "graft hybrids" the author takes up Baur's standpoint that all those which have so far been observed or have been produced experimentally are of the nature of periclinal chimaeras, and in view of recent researches this is a justifiable attitude to take. Since the author refers to some views I have expressed on the subject of graft hybrids I should like to take this opportunity of stating, that like Haberlandt and as a result of the latter's careful investigations of the seedlings of *Crataego-mespilus Asniersii*, I have had to modify my former views of its nature. I now regard it as undoubtedly a periclinal chimaera with an epidermal covering of *Mespilus* cells. Very likely *Crataego-mespilus Dardari* also possesses a chimaeral structure.

With regard to the shape of the epidermal cells of the *Crataego-mespili* which had led me to infer that these graft hybrids were not of chimaeral structure, the statement made by Neilson Jones that "the shape of the epidermal cells may therefore depend in some cases on the character of the growth of the cells below" has been disproved as far as *Crataego-mespilus* is concerned by a recent investigation by Haberlandt (1934), which was probably not available to the author before the publication of the book under review. Similarly he was not able to include in his account the latest of Haberlandt's contributions to the subject in which he shows that while the epidermal cells of the sun leaves of *Crataego-mespilus Asniersii* have straight walls those of the shade leaves have wavy outlines like those of the Medlar. And the same applies to the hawthorn in which the same difference occurs in sun and shade leaves respectively.

Neilson Jones' explanation of the curious character of the leaf of *Pirocydonia* with its serrate tip and smooth base is probably correct, the tip being formed exclusively of pear tissue. This graft hybrid would then be a chimaera with two external layers of pear cells and the leaf tip would be formed mainly if not exclusively from epidermal and subepidermal cells. This explanation

had occurred to me too, and I have been waiting for suitable material to study the development of the leaf.

With regard to *Solanum Darwinianum*, which Winkler regards or regarded as a true graft hybrid, i.e. a burdo, the author is of the opinion that it is more likely to be a periclinal chimaera with a tetraploid tomato core. In the absence of further information about this plant this is probably the most reasonable view to take as both Winkler and Jörgensen obtained tetraploid tomato plants as a result of grafting. Neilson Jones expresses great doubts as to the possibility of a "burdo" arising by fusion of the nuclei of two vegetative cells. It is true that this is very unlikely to occur in the case of nuclei with such a divergent number of chromosomes as is found in the tomato and nightshade respectively, and indeed we have no known case of the fusion of nuclei of vegetative cells of two plants with the same number of chromosomes, though the occurrence of tetraploid plants seems to indicate such a possibility. Perhaps if Winkler's experimental methods were to be undertaken with two dioecious plants, one male and the other female, and possessing the same number of chromosomes, a fusion of vegetative cells might be obtained.

Students will be grateful to the author for having included a substantial account of Krenke's experimental work on the chimaeras of *Solanum lycopersicum* and *S. memphiticum*, as this author's book recently translated into German is not readily accessible and his work is of importance. Similarly Neilson Jones has rendered his account more complete by dealing with the supposed transference of immunity from stock to scion, put forward by Kostoff.

In discussing the white over green chimaeras of *Pelargonium zonale* the author gives Baur's explanation of mosaic inheritance of green and white tissues and the fact that potential green or white pro-plastids may exist in both the pollen grain and the egg cell as demonstrated by Ruhland and Wetzel for the lupin. But though he indicates that that variegation may also be due to a virus disease he does not emphasise the probably very material effect of the plasmon on the developing plastids, particularly if the plastids find themselves in a protoplasm differing from that with which they have been previously associated as in the case of hybrid plants. Reference might also have been made to the suggestions of Stomps and Demerec that variegation might be caused by a labile gene which might undergo mutation.

The author has rightly devoted a special chapter to the anomalous and puzzling chimaeral types in *Hydrangea* and *Pelargonium* and he has advanced our understanding of them by suggesting that in *Hydrangea hortensis variegata* and in the *Pelargonium* known as "Golden Brilliantissima" the structure of the plant is a periclinal chimaera with a white subepidermal layer, but both a green epidermis and a green core. Though further investigation will be needed to establish this suggestion, it is a good working hypothesis in that it does explain the peculiar features of the foliage of these two plants and of the results of breeding experiments. The figure illustrating the probable arrangement of green and white tissues in these two forms and in other periclinal chimaeras of *Pelargonium* is clear and helpful.

The author has adopted the older and I think the preferable spelling of chimaera, but I confess that the adjectival termination "eous," as being more in harmony with the usual botanical terminology, would be more pleasing than the adjectives monochlamydius and dichlamydius used by the author. This is however a very minor criticism, and I feel sure that *Plant Chimaeras and Graft Hybrids* will fulfil the purpose the author has set before him. Students of botany will find it a very stimulating book, as the author indicates quite a number of topics on which further investigations are needed. They will be grateful too for the very explicit account of a subject which has of late grown in importance and upon which an extensive literature embodying very conflicting views has been published. The more important publications bearing on the subject are given in a bibliography with which the book concludes.

La Mutation chez les Orchidées. Par L. REYCHLER. Pp. 164 and 48 plates. Bruxelles: Goemaere. 1928.

M. Reychler is a Belgian orchid grower who has devoted the last twenty years or more to experiments on the hybridisation of orchids. In this volume are given some of the major results of his work. The facts are presented in a series of extremely beautiful half-tone plates of the orchid flowers of the parents and progeny of the crosses. The book does not pretend to scientific status and little information is given except that shown by the photographs. For this reason, together with the unknown genetic constitution of the parent forms and the lack of Mendelian analysis, the first part of the volume will have only a general interest to scientists. The latter part of the book is, however, conspicuous for some extremely striking evidence for phenomena which can only be interpreted as due to telegony. The case is briefly this: that in a cross made between two species of orchid, *a* and *b*, if *a* is uniformly used as the seed parent then year by year the flowers produced by the tubers of the parent plant *a* become like those of a hybrid between *a* and *b*. M. Reychler shows that the phenomenon cannot be due to the act of fertilisation in itself, by experiments in self-pollinating clones both of *a* and *b*. The progressive changes in flower form, induced merely by using species *a* as seed parent in crosses with species *b*, are numerous and striking. They involve large changes in the size, shape, marginal frilling and colour of the labellum and similar changes in the other perianth leaves including differentiation between the two whorls. It is a great pity that the experiments have not been adequately recorded, for they have, as M. Reychler recognises, the deepest significance for both scientists and horticulturists. If the reality of these effects as phenomena of telegony can be established, no plant which has been used as seed parent will afterwards be admissible as expressing merely its inherited characteristics. These experiments were largely carried out with "mutant" species of *Cattleya*, and it is very much to be hoped that they will be repeated scientifically both with these forms and with other species and genera of plants.

H. GODWIN.

Deutsche Waldbäume und Waldtypen. Von W. SCHOENICHEN. Pp. 208 with 41 diagrams in the text and 10 plates. Jena: G. Fischer. 1933. Rm. 14—Geb. Rm. 15.50.

In recent years the increasing popularity of ecological investigation has added enormously to the information already acquired by botanists and foresters, of the native trees and woodlands of Germany, and Dr Schoenichen has undertaken the collection and presentation of this large body of profoundly interesting data. The following species are dealt with: *Taxus baccata*, *Pinus cembra*, *P. montana*, *P. silvestris*, *Picea excelsa*, *Abies alba*, *Larix europaea*, *Fagus silvatica*, *Carpinus betulus*, *Quercus robur*, *Q. sessiliflora*, *Alnus glutinosa*, *A. incana*, *A. viridis*, *Tilia cordata*, *T. platyphyllos*, *Ulmus campestris*, *U. montana*, *U. effusa*, *Acer pseudo-platanus*, *A. platanoides*, *A. campestre*, *Fraxinus excelsior*, *Populus tremula*, *P. nigra*, *P. alba*, *Betula verrucosa*, *B. pubescens* and various species of *Salix*. The treatment varies according to the importance of the different species and the amount of information available, but in most cases there is a description of the geographical and altitudinal range of the species in Germany and in Europe, a full description of the climatic and habitat preferences, of economic status and forest history, of the woodland types which include the species, and of the major plant communities which have been described for these woodlands. The book is well illustrated by diagrams and half-tone illustrations. The scope and treatment guarantee a work of great interest and value, but the production is nevertheless a disappointing one. The

diagrams showing the European distribution of various trees would be invaluable could they be trusted, but the British data are often wrong and lead one to doubt the validity of the other limits shown. Thus the beech forest limit in Britain is shown to cross the Scottish lowlands, and the only authority quoted is Erdtman, and Tansley and Watt are not mentioned though their views were published even before the appearance of *Die Buchenwälder Europas* in 1932. The hornbeam limit is also wrong and Miller Christie is not mentioned. *Tilia platyphyllos* is not shown native in Britain at all.

Along two other lines at least the book could also have been much better. The very considerable amount of information about the post-glacial forest history of Germany made available by pollen-analysis in the last few years deserves a good deal more than the fragmentary references given here. This defect the author might have remedied; the other is largely out of his control and this lies in the entire neglect of the dynamics of the communities described. Even where climatic climax communities have been established investigation of the seres leading up to them will give invaluable information as to the significance of the various facies of the climax communities. And when the woodlands are evidently not near the climax condition, as in the case of the alder, willow and birch brush woods, to neglect successional relations is to obscure the meaning of all the major ecological phenomena. The two chief types of alder wood, the "Erlensumpfmoor" and the "Erlenstandmoor," are indeed said to have different origins; it is not recognised that in their history must lie most of the keys to their present structural differences. Without this information it must remain uncertain what are the affinities of these alder woods with the alder woods of the rest of Europe, of, for instance, the delta of the Danube or the Norfolk Broads.

Despite the shortcomings we have indicated, Dr Schoenichen's book does contain a great deal of very valuable information and will serve, especially by its constant references to a very wide German bibliography, as a ready means of facilitating comparison between the German woodlands and those of the neighbouring European countries.

H. GODWIN.

The Fresh-Water Algae of the United States. By GILBERT M. SMITH.
9 × 5½ in. Pp. xi + 716. 449 figs. McGraw-Hill Book Company,
Inc., New York and London. 1933. Price 36s.

Prof. Smith's imposing volume is a text-book of freshwater algology as well as a descriptive catalogue of the genera found in the United States. He treats his subject on the modern lines made familiar to English students by the writings of Prof. Fritsch. A brief introductory chapter explains these modern views on the phylogeny and classification of pigmented protista and discusses the ecology and periodic activity of these organisms. There are also notes on the collection and preservation of freshwater types. In the remainder of the book the chief classes recognised by Pascher are dealt with in succession. For each class a very useful and up-to-date general description is followed by a systematic account of the genera which occur in the United States. The distinguishing characters of orders and families are set forth clearly, and there are artificial keys to the genera. For genera with fewer than ten species in the United States, "there is a brief characterisation of the different species." At the end of the book is a key to all the genera described. About two-thirds of the 449 figures are original. The book has the usual form of McGraw-Hill publications, and is therefore well printed on good paper and well bound, but is much too expensive in this country.

It is admitted that the demonstration of a phylogenetic connection between flagellata and algae might constitute "an argument for placing all pigmented

protista among the algae or protophyta." The argument is nevertheless rejected because it necessitates "the inclusion, with the algae, of many forms ... more animal-like than plant-like." Only those classes are included, therefore, of which there are representatives with "a truly algal organisation." There is here an unfortunate ambiguity in the use of the term "alga," and a more serious restriction of the scope of the book. Prof. Smith is, moreover, inconsistent in admitting the slender claims of Euglenophyceae to inclusion, while rejecting those of Cryptophyceae, although there are at least three genera of this class with algal characteristics (including *Phaeococcus*, mentioned on p. 187) and none with purely holozoic nutrition. A more logical position would surely be to include all pigmented protista, giving a general description of the various classes to which they belong, but excluding holozoic members from the special sections. The Cryptophyceae and Desmokiaceae could then be included and such forms as the Chrysophycean *Physomonas*, with no chromatophores and "nutrition entirely holozoic" could be omitted from the detailed descriptions.

The most interesting point of systematic treatment is the sub-division of the Chlorophyta. Fritsch is followed, as against Pascher, in recognising only one class, Chlorophyceae, the Conjugatae, called Zygnematales, being placed as an order of equal status with eight others. The Ulotrichales and Chaetophorales of Fritsch are combined under the former name, but with Ulvales and Schizogoniales (including *Prasiola*) removed as separate orders. Chlorococcales, with no vegetative cell divisions, are reasonably regarded as off the main line of evolution of the filament, and the ordinal status of the Tetrasporales is emphasised because they are much more probably on this line, linking Volvocales with Ulotrichales. It is true that they have vegetative cell division, but the daughter cells seem always to secrete whole new walls, the parent cell wall gelatinising or rupturing. Types ancestral to Ulotrichales might be expected to exhibit their constant characteristic of a utilisation of the parent wall material in the construction of the new cells. It is interesting in this connection that a phragmoplast, as Prof. Smith notes, has been recorded for *Tetraspora*, *Eremosphaera* and *Spirogyra*, so it is not, as might be expected, the presence or absence of this feature which determines the distinction between the non-motile colony and the filamentous or parenchymatous form. It would be valuable to have a detailed investigation of cell division in a wide range of algae, for it is thus that the precursors of the filamentous forms will probably be discovered. They might be amongst Prof. Smith's Tetrasporales: an order that can include *Malleochloris*, *Prasinocladus* and *Tetraspora* is a promising hunting ground.

Of thirteen genera of Heterokontae, fourteen of Chrysophyceae and thirty-seven of Bacillariaceae listed in British Freshwater Algae (West and Fritsch) as occurring in Britain, ten, ten and thirty-five respectively are described in this book, with several others not yet recorded for Britain. These figures give some idea of the value of the book to workers wishing to identify organisms collected in this country. They are also interesting as showing the similarity of algal floras in widely separated countries of comparable climate.

A. R. CLAPHAM.

INDIAN AGRICULTURAL RESEARCH
INSTITUTE LIBRARY, NEW DELHI.

[illegible]

GIPNLK—H-40 I.A.R.I.—29-4-55—15,000